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THE LIFE-HISTORY OF *PUCCINIA RUELLIAE* (B & Br) ON *RUELLIA PROSTRATA* POIR.

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Received March 11, 1947

(Communicated by Dr. T. S. Sadasivan, M.SC., PH.D., F.A.SC.)

SYDOW (1904) has recorded five species of *Puccinia* on the genus *Ruellia*. He divided these into two groups one having verrucose-walled teliospores and the other smooth-walled. Coming under the former are *P. longiana* on *R. tuberosa*; *P. lateripes* (Berk. and Rav.) on *R. ciliosa*; *P. ruelliæ-bourgæi* (D. and H.) on *R. bourgæi* and *P. ruelliæ* on *R. strepens* and *R. prostrata*. *P. paranahybæ* which has smooth-walled teliospores is recorded on *R. longifolia*. The rust on *R. prostrata* was from Ceylon while the others were from the Americas. Butler and Bisby (1931) have mentioned the occurrence of *P. lateripes* on *Ruellia* spp. at Pusa (Bihar) and *P. ruelliæ* on *R. longifolia* (at Cawnpore) and *R. prostrata* in various parts of India. Arthur (1934) has given six species of *Ruellia* serving as hosts for *P. ruelliæ* in the U.S.A. Further he has reduced *P. lateripes* and *P. ruelliæ-bourgæi* to the status of synonyms of *P. ruelliæ*. This appears to be the correct view as there are no significant differences between the different species belonging to the group possessing verrucose teliospores.

Kellerman (1903) conducted successful infection experiments with *P. lateripes* on *R. strepens*. Arthur (1906) was able to produce infection on *R. ciliosa* and *R. strepens* with teliospores of *P. lateripes*. *P. ruelliæ* is very common on *R. prostrata* in and around Coimbatore in South India. It is a macro-cyclic and autœcious rust, all the stages being formed on the same host in the course of one and the same season. The succession of the different spore forms and their methods of development were closely studied. Advantage was taken of the occurrence of all the spore forms to follow the sequence in the development of the different stages by inoculation experiments. The present studies were carried out on *R. prostrata* and the results are recorded in this communication.

R. prostrata is a common perennial weed in South India which persists throughout the year in shady situations, but the rust is in evidence at

Coimbatore mainly from August to March of the succeeding year. During the south west monsoon in July and August the weed puts on fresh growth. The uredio and telial stages are seen in profusion at the beginning of this period. The lower leaves of the plant are the first to be infected. These sori are usually not found on the youngest leaves.

Uredia.—These are amphigenous and brown in colour. The sorus develops sub-epidermally. Later the epidermis is lifted up and finally ruptured. The hyphæ of the fungus are intercellular, sending prominent twisted haustoria into the host cells. The uredial primordium forms below the epidermis and from this the urediospores are produced. These spores are stipitate, spherical to sub-globose, echinulate and yellowish brown in colour. Two equatorial germ pores are seen. Two nuclei are distinctly visible. The spores measure $22 \times 23\,\mu$.

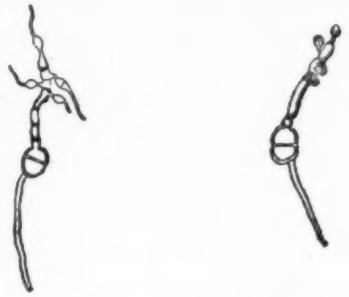
FIG. 1. Young urediospores ($\times$ 300).

FIG. 2. Teliospores ($\times$ 250).

Telia.—Soon after the formation of uredia, telia are formed often associated with uredia. Teliospores may develop either in urediosori mixed with urediospores or in separate sori. These are amphigenous, confined to the leaf-blades but more numerous than the urediosori and scattered all over the surface. They are dark chestnut brown, surrounded by the whitish flakes of the ruptured epidermal tissue. The development of the telia is sub-epidermal as in the uredia. Teliospores are pedicellate, the pedicel being hyaline and attached to the spore variously, *i.e.*, to the base or obliquely to the side. The pedicel breaks easily leaving a portion of varying length persistently attached to the spore. Teliospores are two-celled chestnut brown, thick-walled, verrucose, sub-globose with rounded ends, slightly constricted at the septum and with one germ pore in each cell. The germ pore of the apical cell is at the top while in the lower cell it is placed variously. The spores measure $24 \times 36\,\mu$.

Teliospores germinate readily without a period of rest. As a matter of fact, fresh spores collected in August, November and December exhibited a higher percentage of germination than those from specimens collected in

March. Maneval (1922) found that the time required for the germination of teliospores varied with the period in which germination tests were made. It is less than two days in May, less than eight days in December, but over eighty days in September under conditions prevailing in Columbia. Under Coimbatore conditions, germination is evident in 12 to 24 hours when fresh spores are floated on drops of water on slides and kept inside a moist chamber. Germination was visible in hanging drops also in the spores which floated near the margin of the drops, while those which were immersed in water did not germinate. This indicates the necessity for aeration for teliospore germination. The promycelium emerges out through the germ pore. It is stout, hyaline and four-celled, the septa developing in the upper half of the promycelium. From each cell a sterigma is developed and on this an oval or round hyaline basidiospore is formed. Basidiospores commence germination even while they are attached to the basidium.



a *b*

FIG. 3. (*a*) Germinating teliospore showing the germination of basidiospores (×100). (*b*) Germinating teliospore (×100).

EXPERIMENTS AND OBSERVATIONS

Inoculations were made on healthy seedlings of *Ruellia prostrata* specially raised for the purpose and free from rust. Fresh teliospores were removed from sori and placed on drops of distilled water on the leaves or a suspension of fresh teliospores in sterilized water was brushed with a sterile camel-hair brush over the surface of the leaves, petiole and stem. The pots containing the seedlings were kept covered over with bell-jars to provide favourable conditions for infection and to prevent natural infection. Suitable control plants were also kept under similar conditions. Ten to twelve days after inoculation, groups of pycnia were evident on the inoculated leaves, petioles and stem. Younger leaves, petioles and stems were readily infected, while mature lower leaves or older portions of the stem

failed to take infection. This is explained by the fact that basidiospores are able to penetrate only younger tissues as the entry is effected by piercing the epidermal cell wall. On the leaf-blades circular swollen areas develop which are convex either towards the upper or lower surface with a corresponding depression on the opposite side. The areas become studded with very minute red or yellowish brown pycnia on both sides. Pycnia are formed in abundance on the portions of the petioles and stem which are swollen as a result of hypertrophy. They are globose or oval, sub-epidermal, paraphysate and measure on an average $110\times 190\,\mu$. The paraphyses and the spore stalks are elongated, uninucleate and light orange in colour. From the apex of each conidiophore, oval to oblong pycniospores ($4{\cdot}5\times 6{\cdot}0\,\mu$) are formed. These are hyaline or light pink in mass and float in the nectar which collects at the mouth of the pycnium. The paraphyses are prominent and project out of the ostiole. Aecia develop in the midst of groups of pycnia found on the leaf veins, petioles and swollen portions of the stem. The æcia are not formed on the interveinal portions of the leaf-blade though pycnial groups are present in these areas. The infected portions become swollen due to the hypertrophy of the tissues. In the leaf, the palisade cells of the mesophyll become enlarged. In the petiole and the stem the cortical cells are very much enlarged in the infected portions and contribute to the formation of the swellings. In severe cases of infection, clusters of branches like 'witches broom' develop from the nodes and numerous æcia are studded on the stem of these abnormal branches (Plate I, *c*).

a *b* *c*

FIG. 4. (*a*) Pycniospores (× 150). (*b*) Section of a portion of a young æcium (× 300). (*c*) Germinating æciospore (× 150).

Aecia.—These are cupulate with a nearly cylindrical more or less white peridium with jagged edges, which soon become recurved. The æciospores in mass are coloured deep brown. They are elliptic to irregular and thick-walled, the thickening being more pronounced at the apex and base,

prominently verrucose and measure $22 \times 30\,\mu$. One conspicuous germ-pore is present.

The primordium of the æcium is first composed of a plectenchymatous mass of hyphæ formed three or more cell layers below the epidermis and which force the host cells apart. The host cells separate and in the resulting space the æcium develops. Its development is similar to what is observed in many of the cupulate æcia. The binucleate nature of the cells is evident in the basal cells of the hymenium and also in many of the hyphal cells found much below the hymenium (see Fig. 4 *b*). The peridium is made up of one layer of cells which forms a continuous envelope extending from the sides and arching over the young æcium. Later, the host tissue is pushed up and eventually ruptured exposing the æcium and the æciospores. The æciospores are developed from the basal cells in chains. The æciospores alternate with intercalary cells which shrivel and disintegrate, thus facilitating the dismemberment of the æciospores from the chains. Aeciospores are capable of immediate germination. One germ tube is produced from each spore which grows out through the germ pore. Viability of the æciospores is gradually lost with age. Fresh æciospores germinate in twelve hours but æciospores from specimens which had been kept between drying sheets and stored in envelopes at laboratory temperature (26–28° C.) for three months did not exhibit any signs of germination even after two days under optimum conditions. In December, suspensions of fresh æciospores were brushed on the surface of leaves of healthy *Ruellia* seedlings and these were covered over with bell-jars to provide humid conditions. In fifteen to twenty-two days uredia developed on the inoculated leaves. Unlike the pycnia produced from basidiospores, the urediosori were formed only on the older lower leaves and failed to infect younger leaves at the apices of the shoot. The entry of germ tubes from æciospores being through the stomata, the older leaves are readily infected. In the course of another week, teliosori were also observed on the same leaf. The control plants were free from infection.

The above studies have shown that *P. ruelliæ* is a macro-cyclic, autœcious eu-form of rust exhibiting 0, I, II, and III stages. Starting with the teliospore the life-history has been followed up through all the other stages. During these studies it was found that during one season this rust can complete two or more cycles on this host.

Summary

The life-history of *Puccinia ruelliæ* on *Ruellia prostrata* was studied by inoculation experiments. It is a macro-cyclic and autœcious rust, all the

stages being formed on the same host in one and the same season. The succession of the different spore forms and their method of development were closely studied. Advantage was taken of the occurrence of all the spore forms to follow the sequence in the development of the different stages by inoculation experiments.

ACKNOWLEDGMENTS

My thanks are due to Mr. T. S. Ramakrishnan, Assistant Mycologist, Agricultural College and Research Institute, Coimbatore, for helpful criticisms and suggestions. I am also grateful to Mr. K. M. Thomas, Government Mycologist, Agricultural College and Research Institute, Coimbatore, for having gone through the manuscript and rendering necessary help to carry out this piece of investigation.

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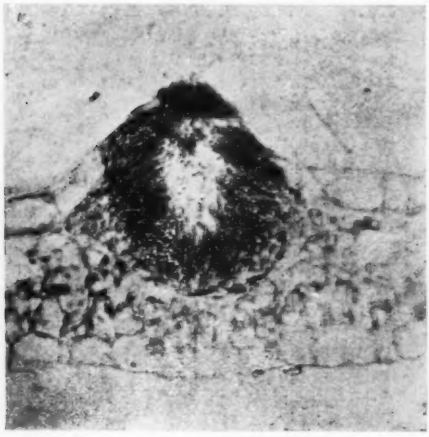

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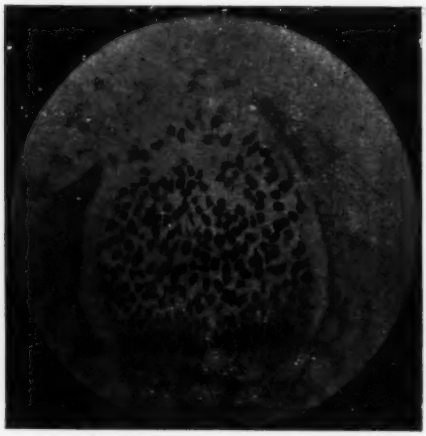

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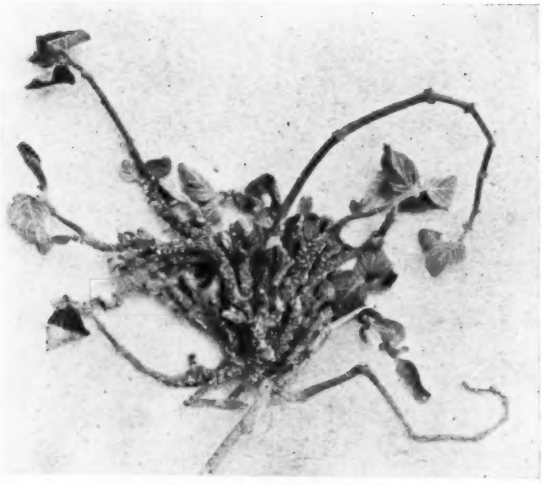

C

A. Section through a pycnium showing paraphyses and the pycniospores.
B. Section through an æcium showing the hymenial layer, peridium and the æciospores.
C. Showing cluster of branches like "witches broom" and the numerous æcia studded on the branches.

Pl. 1

ADDITIONS TO FUNGI OF MADRAS—III*

By T. S. Ramakrishnan and K. Ramakrishnan
(*Mycology Section, Agricultural Research Institute, Coimbatore*)

Received May 26, 1947
(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

(10) *Physalospora pterolobii* Ramakrishnan, T. S. and K. sp. nov.

Spots circular, isolated, epiphyllous, yellowish green with black centre; *perithecia* one to four in a spot, immersed in the tissue, deep-seated, globose, ostiolate, 290 × 266 μ (259–335 × 222–315 μ), peridium of two to three layers of dark brown cells, thicker at the apex near the ostiole; *asci* cylindric-elongate, 107 × 14 μ (85–136 × 9–19 μ), produced from the base and the sides, 8-spored; *ascospores* oblong, one-celled, hyaline, uniseriate, 13 × 5 μ (11–18 × 4–7 μ); paraphysate, *paraphyses* filiform; *pycnidia* immersed in the tissue, associated with the perithecia, globose, containing minute, hyaline, rod-shaped, spores. The pycnidium resembles a spermagonium.

On living leaves of *Pterolobium indicum* A. Rich., Kallar (Coimbatore district) 9–X–1946, T. S. Ramakrishnan.

Maculæ orbiculares, epiphyllæ; *perithecia* 1–4 per maculam, subepidermia, globosa, ostiolata, paraphysata, *paraphyses* filiformes; *asci* elongati-cylindrici, 107–14 μ, octosporiati; *ascosporidia* oblongata unicellata, hyalina, uniseriata, 13 × 5 μ; *pycnidia* subepidermia, globosa, prope perithecia, *pycnidiosporidia* minuta, hyalina, baculoformia.

In vivis foliis *Pterolobi indici* A. Rich., Kallar (Coimbatore) 9–X–1946, T. S. Ramakrishnan.

The leaf is thickened at the region of infection due to the enlargement of the mesophyll cells.

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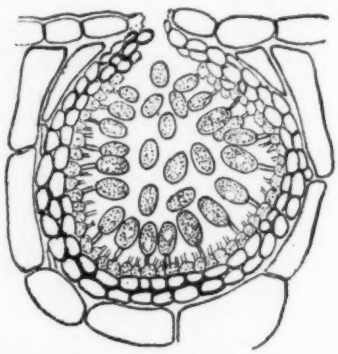
* Part I of this series was published in *The Proceedings of the Indian Academy of Sciences*, January 1947, **25**, No. 1.

Part II in the same Journal, June 1947, **25**, No. 6.

The type specimens have been deposited in the Herbarium of the Government Mycologist, Coimbatore, and Herb. Crypt. Ind. Orient., New Delhi.

(11) *Physalospora heterostemmæ* Ramakrishnan, T. S. and K., sp. nov.

Perithecia epiphyllous, gregarious, groups scattered, 4–10 or more in each group, innate, extending up to the spongy parenchyma, erumpent, dark-brown to sepia, ostiolate, peridium of two to three layers of thick-walled dark-brown, polygonal cells, 178× 170 μ (155–203× 150–185 μ), paraphysate, *paraphyses* linear and septate; *asci* clavate, hyaline, 8-spored 74× 13 μ (64–93× 7–15 μ); *ascospores* irregularly uniseriate, one-celled oblong, hyaline, 15× 7 μ (11–19× 6–9 μ); *pycnidia* of two kinds, occurring near the perithecia; one type of pycnidium immersed, globose, 111× 111 μ ostiolate, with a dark brown, two to three layered peridium; *pycnidiospores* oval, hyaline, one-celled, and resemble those of *Macrophoma*; second type of pycnidium also globose, immersed, in the tissue, producing large numbers of minute, hyaline, rod-shaped pycnidiospores. This structure resembles a spermagonium (Plate II, *c*).



TEXT-FIG. 1. Pycnidium (*Macrophoma stage*) × 400.

On living leaves of *Heterostemma tanjorense* W. and A., Kallar (Coimbatore district), 9–X–1946, T. S. Ramakrishnan and K. Ramakrishnan.

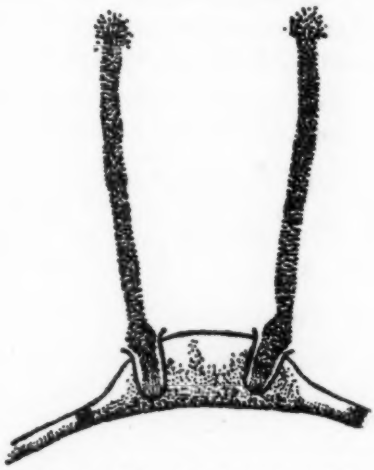
Perithecia epiphylla, gregaria, subepidermia, erumpentia, ostiolata, 178× 170 μ; *asci* clavati, hyalini, 74× 13 μ, octosporiati; *ascosporidia*, irregulariter uniseriatea, oblongata, hyalina, 15× 7 μ; *paraphyses* adsunt murus asci gelatinous in aquæ; 2 genera pycnidium, 1) immersa, globosa, *pycniosporidia* hyalina, ovalia unicellata, 2) globosa, *pycniosporidia* minuta, hyalina, baculo-formia.

In vivis foliis *Heterostemma tanjorensis* W. and A., Kallar (Coimbatore district) 9–X–1946, T. S. Ramakrishnan *et* K. Ramakrishnan.

The asci start from the base of the perithecium and when mounted in water exhibit intense gelatinisation of the wall and consequently a clear translucent area is seen between the spore and the outer wall of the ascus.

(12) ***Kernia lauricola*** Thirumalachar

This was collected on two hosts belonging to the Lauraceæ-*Phœbe paniculata* Nees. and *P. Wightii* Meissn., from several places in the Nilgiris (Ootacamund, Coonoor and Naduvattam). Only the telial stage was available. The telia are columnar and are produced in a circle from the margins of small circular swellings on the lower surface of the leaves (Plate *a*). Six to twelve such columns are produced in each ring. The telium takes its origin from a layer of long parallelly arranged hymenial cells at the bottom of a cup-like depression. The teliospores are stipitate, two-celled with long stalks. The columnar structure is produced by the plaiting together of the stalks of the released teliospores. The teliospores measure 35× 22 μ (26–42× 16–28 μ). The two cells of the teliospores are almost equal in length. They are deep chestnut brown in colour and have smooth walls. There is a constriction between the two cells. This rust closely resembles the species recorded by Thirumalachar (1946) on *Litsea* sp.



TEXT-FIG. 2. Telia of *K. lauricola* × 100.

(13) ***Cercospora adinæ*** Ramakrishnan, T. S. and K., sp. nov.

Spots hypophyllous, without any definite outline, forming irregular often confluent ochraceous-orange patches, involving much of the leaf surface; mycelium internal, septate; *conidiphores* hypophyllous, densely tufted, emerging through the stomata, branched or unbranched, filled with deep orange contents and repeatedly geniculate at the apices; *conidia* elongate, broad below the middle, and tapering towards the apex, base

flattened, apex rounded, straight or curved, 3–7 septate, 54–84 × 4–7 μ, contents hyaline to orange.

On living leaves of *Adina cordifolia* Hook., Walayar (Malabar district) 31–XII–1936, T. S. Ramakrishnan and K. Ramakrishnan.

TEXT-FIG. 3. Conidiophores and conidia of *Cercospora adinæ* × 400.

Panni hypophylli, silacei-lutei colores, *conidiophora* dense fasciculata, emergentia per stomata, septata, contents dense lutea, *conidia* elongata, obclavata, recta vel curva, 3–7 septata, hyalina vel lutea, 54–84 × 4–7 μ.

In vivis foliis *Adinæ cordifoliæ* Hook. Walayar (Malabar district) 31–XII–1946, T. S. Ramakrishnan *et* K. Ramakrishnan.

This fungus does not form definite spots on the leaves and often the incidence of the infection cannot be detected from the upper surface. Since this fungus is found to be different from the others recorded on plants belonging to the family Rubiaceæ it is described as a new species.

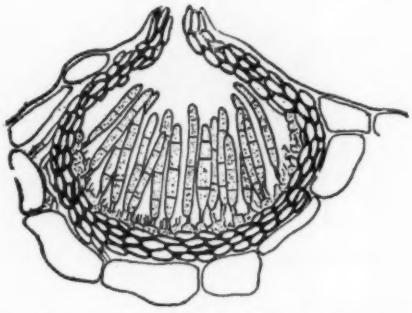
(14) *Septoria erythrinæ* Ramakrishnan T. S. and K., sp. nov.

Spots numerous, small, angular, bounded by veinlets, light green in colour; *pycnidia* hypophyllous, 2–5 in a spot, black, subepidermal, immersed in the tissue, slightly erumpent, globose, ostiolate, 150 × 90 μ, with a peridium of two to three layers of brown cells; *pycnidiospores* long, cylindrical, 44 × 4 μ (36–58 × 4–6 μ), straight, three-septate, hyaline produced on very short stalks.

On living leaves of *Erythrina* sp. Kallar (Coimbatore district) 9–X–1946 T. S. Ramakrishnan and K. Ramakrishnan.

Macuale parvæ, angulares, leviter virides; *pycnidia* hypophylla, 2–5 per maculam, nigra, subepidermia, globosa, ostiolata, 150 × 90 μ; *pycniosporidia* cylindrica, recta, triseptata, hyalina, brevipedicellata, 44 × 4 μ (36–58 × 4–6 μ).

In vivis foliis *Erythrinæ* sp. Kallar (Coimbatore district) 9–X–1946; T. S. Ramakrishnan *et* K. Ramakrishnan.



TEXT-FIG. 4. Pycnidium of *Septoria erythrinæ* × 400.

The spores come out of the ostiole in the form of thread-like whitish masses. When examined with a lens these can be readily made out in fresh specimens as white peg-like projections from the pycnidia ('spore horns').

Phlyctæna brunneola (Berk.) Sacc. (*Septoria brunneola* Berk.) has been described on dead branches and stem of *Erythrina crista-galli*. Saccardo does not give the measurements of the pycnidiospores or the pycnia. The fungus under study is however found parasitic on living leaves of *Erythrina* sp. and is not a *Phlyctæna*. It is undoubtedly new to science and is proposed as a new species.

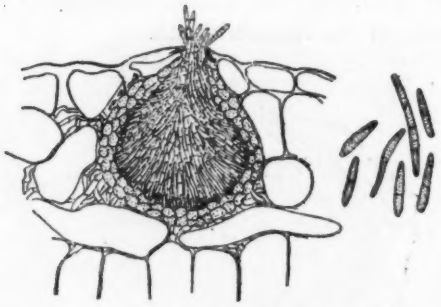
(15) *Septoria thespesiæ* Ramakrishnan T. S. and K., sp. nov.

Spots circular, amphigenous, isolated or confluent, 4–15 mm. in diameter, upper surface blackish brown with grey centre and lower surface sepia coloured; *pycnidia* globose, innate, immersed in the tissue, numerous in the spot, 88 × 93 μ (74–96 × 63–111 μ), peridium of two to three layers of small cells, *pycniospores* straight cylindrical with tapering ends 2–6 septate, hyaline, borne on very short stalks, 28 × 4 μ (9–37 × 2–4 μ).

On living leaves of *Thespesia populnea* Cav. Coimbatore, 18–ii–1947, T. S. Ramakrishnan and K. Ramakrishnan.

Maculæ orbiculares, amphigenæ; *pycnidia* globosa, subepidermia, ostiolata; *pycniosporidia* recta, cylindrica, cum terminis angustitatis, 2–6 septata, hyalina, brevipedicellata, 28 × 4 μ (9–37 × 2–4 μ).

In vivis foliis *Thespesiæ populneæ* Cav. Coimbatore, 18–ii–1947, T. S. Ramakrishnan *et* K. Ramakrishnan.



TEXT-FIG. 5. Pycnidium of *Septoria thespesiæ*, pycnidiospores × 300.

This is very common on *Thespesia populnea* throughout the province all through the year. The spots become almost black on old yellow leaves. The pycniospores come out in masses as ' spore horns ' through the ostiole of the pycnidium.

ACKNOWLEDGMENT

The authors are grateful to Dr. B. B. Mundkur of New Delhi for going through the manuscript critically and to Mr. K. M. Thomas, Government Mycologist, Coimbatore, for his constant interest and encouragement. We are also thankful to Mr. M. S. Balakrishnan, Research Fellow, for making some of the drawings, and to Rev. Fr. M. Singarayar of St. Joseph's Seminary, Coimbatore, for the Latin translations of the diagnoses.

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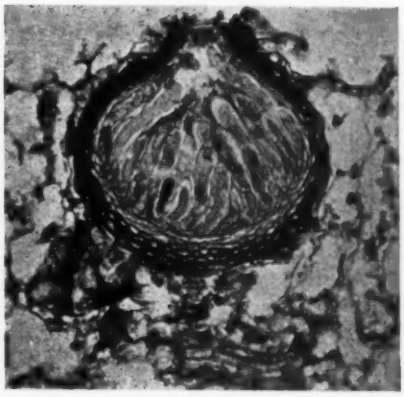

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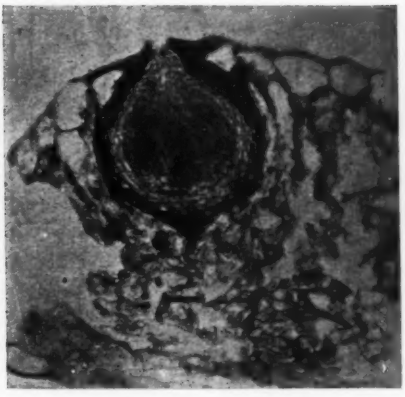

c

a. Leaf of *Phœbe paniculata* showing the telial columns of *Kernia lauricola*.
b. Section of a perithecium of *Physalospora heterostemmæ*.
c. Section of a spermagonium of *Physalospora heterostemmæ*.

STUDIES IN THE ANATOMY OF SUGARCANE STALK

I. Chewing Canes

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I. Introduction

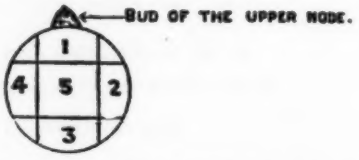
The study of chewing canes was taken up because of their economic importance in urban areas. In the vicinity of large towns, there is always a great demand for them, and a small farmer with irrigation facilities and adequate manuring can get a remunerative return for his investment if he puts in the market, a really suitable variety for sale. Apart from the sweetness, the thickness and the colour of the cane, the ease with which it can be peeled and crushed by the human jaw, is the primary consideration desired in a chewing cane. The latter features naturally call for a detailed study of the anatomical make-up of the stalk as a whole to enable differential ratings in respect of the rind and core tissues in different varieties to be adequately assessed. While the internal structure of sugarcane stalk was studied in some detail by Bremekamp (1914) and Artschwager (1925), no serious attempt seems to have been made till recently to work out the comparative anatomy of different varieties and correlate it with features of economic importance. Ueno (1938) demonstrated that rind hardness was closely associated with the number or the size of vascular bundles. Khanna and Panje (1939) found that rind hardness was not a simple function of vascular bundles alone. According to them, the lignification of parenchymatous matrix and vascular sheaths was also a major factor involved in determining the degree of rind hardness. Buzacott (1940) and Rao (1941) showed that anatomical structure of a cane variety as reflected in the rind hardness contributed much towards its resistance to stem-borers.

The present contribution is an attempt to understand what anatomical features are a pre-requisite in a cane stalk to be suitable for chewing purposes.

II. Material and Methods

Three well-known varieties of chewing canes, namely Saharanpur Paunda, Amritsar Paunda and Peshawar Paunda, were selected for this study. The upper half of middle internodes of three representative stalks

formed a sample for each variety. Since it was not possible to get intact, an entire cross-section of so thick a cane, the internode was longitudinally split into four peripheral and one central sectors which were numbered as shown below.



Hand sections from each sector were stained with 1% solution of Safranin in 50% alcohol, and after the usual process of dehydration, were mounted in Canada Balsam.

The following characters were studied:—

A. *Vascular bundles*—

(*a*) *Number per unit area.*—All the vascular bundles occurring in 3 unit-areas were counted in each sector, the field of the microscope being taken as a unit. In order that the counting was done in regions comparable to one another, the edge of the field of microscope, was kept touching the epidermis in sectors 1 to 4 and for the 5th sector, innermost portions were taken. There were 36 countings for rind and 9 for central region for each variety.

(*b*) *Size.*—Because of the wide variations in the size of the vascular bundles occurring in the rind region and the inherent difficulty in selecting them without any bias, it was measured for all of them found in the field of the microscope, for each unit area, in terms of their two axes, radial and tangential, with respect to the cross-section of the cane. In the central region where the variability in their size was rather low, the size of only six per field which amounted to nearly 50–65% of the population, was measured. Thus 358 vascular bundles were measured in the peripheral sectors of the Saharanpur Paunda: 369 for the Peshawar variety and 465 for the Amritsar Paunda. For the central region, 54 observations were made for each variety.

In view of the basic pattern of a quadrilateral figure symmetrical along its radial axis, to which all the shapes of vascular bundles conformed, it appeared that the product of the two axes would give a very reliable index of the size of vascular bundles in different varieties for a comparative study. To test this hypothesis, size of all the vascular bundles in a unit area (1·9 mm. in diameter) in each of the four peripheral sectors of all the three canes in each variety was determined by the following three methods:—

(i) The radial and tangential axes of vascular bundles were measured under microscope in terms of the divisions of eye-piece micrometer, product of the two giving a comparative value of the size.

(ii) Those very vascular bundles were drawn 140 times magnified with the help of a camera lucida. Product of the two longest axes of the figure at right angles to each other and measured to the nearest mm. was taken as an index of the area of the figure.

(iii) The actual area of the figure was measured with a planimeter to the nearest 0·25 sq. cm. As this obviously bore the most accurate relationship to the actual respective areas of the vascular bundles, it was taken as the standard with which the two sets of indices mentioned above had to be compared.

Statistical analysis of these three sets of data for 517 vascular bundles (193, 164 and 160 vascular bundles respectively of Amritsar, Saharanpur and Peshawar Paundas) showed that there existed a very high positive correlation of the order of 0·9546 between (i) and (iii) and 0·9699 between (ii) and (iii).

Similar determinations for 90 vascular bundles taken at random from central sectors, 10 from each gave + 0·8889 as coefficient of correlation between (ii) and (iii) methods. The coefficient of correlation between (i) and (iii) was not determined because of the close correspondence found to exist between this value and that for (ii) and (iii) in respect of the vascular bundles of the peripheral region.

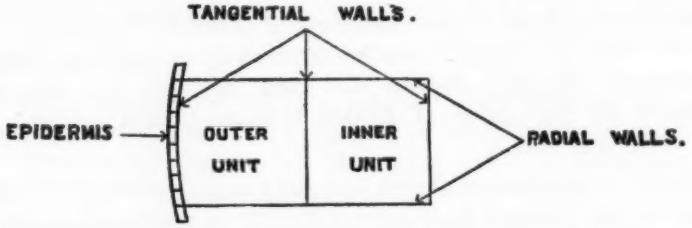
The method (i) followed for determining the size of vascular bundles was, therefore, quite reliable for a comparative study. Besides the method was both quicker and less laborious, thus enabling a large number of measurements to be taken. A further contribution on the subject will, however, be made separately.

(*c*) *Total area* occupied by vascular bundles in a unit area in peripheral region was found by adding the size of all of them and in the case of the central sector by multiplying the average size (calculated from that of six bundles in a particular unit) by their number in that unit.

B. *Parenchymatous cells*—

In a unit area (square in shape), the number of these cells, which obviously, was inversely proportional to their average size and was therefore an index of the latter, was calculated by multiplying the number of cells situated on the two adjoining sides of the square in the case of the 5th sector where total area under vascular bundles in a unit area, was not

significantly different in these varieties. To get the requisite figure for peripheral sectors, where cells rapidly increased in size as one proceeded from the epidermis towards the centre, the average of the number of cells situated on the opposite sides of the square (tangential in respect to the cross-section of the cane) was multiplied with that on the radial. For the outer unit, epidermis formed the outer of the two (tangential as stated above) sides of the square while the inner unit was bounded towards the periphery by the inner side of the outer unit as shown in the diagram below. The area occupied by the vascular bundles did not interfere with such determinations because parenchymatous cells in this region were arranged more or less in layers. Actual counting of cells in a unit area or the determination of cell-size by planimeter did not seem to be a practical proposition.



36 observations each for the outer and inner units, were made in the peripheral region and 9 for the core for each variety.

C. *Thickness of cell-walls*—

This was measured for two adjoining cells in the parenchyma at 0·6 mm. and 1·2 mm. from the epidermis and in the centre. Similarly for the sclerenchyma, it was noted in the sheaths of 2–3 vascular bundles, the biggest of them in the field. In rind, 120 readings were taken at each depth for parenchyma and sclerenchyma and 30 only for each tissue in the core of a variety.

III. Observations and Data

From the foregoing paras, it would appear that the cross-section of a cane stalk was studied more or less completely for all the anatomical features which were responsible for the hardness of a cane. They are listed below:

(A) The number, the size and the total area of vascular bundles in a unit area and the thickness of walls of cells forming their sheaths.

(B) The number of parenchymatous cells in unit area and the thickness of their walls, the former being an index of their size.

Since the total area occupied by the vascular bundles was a function of their number and size, and included these two characters in their entirety, a separate consideration was not given to them as such, thus leaving for critical examination, six characters for rind, *viz.*: (i) the area under vascular bundles, (ii) number of parenchymatous cells in outer unit area and (iii) in inner unit area, (iv) the thickness of cell-walls in parenchyma at 0·6 mm. and (v) at 1·2 mm. and (vi) the thickness of cell-walls in the vascular sheaths, and four characters for the storage tissue, namely: (i) total area of vascular bundles, (ii) number of parenchymatous cells in unit area and (iii) thickness of cell walls in parenchyma and (iv) in sclerenchyma.

Rind.—In the formation of rind, the Amritsar Paunda showed the greatest development of five characters, *viz.*, the total area of vascular bundles, the thickness of cell-walls of sclerenchyma, and parenchyma at 0·6 mm. depth and the number of parenchymatous cells in both the outer and inner units (Table I) in all of which except the last named character it was significantly different from the other two varieties at 1% level (Plate III, Figs. 1–3). The difference between this variety and the Peshawar Paunda in respect of this character was significant at 5% level only. The size of the parenchymatous cells being inversely proportional to their number in unit area, the difference in their size was clearly visible where their number in two varieties was significantly different, the higher the level of significance, the more pronounced the difference in their size. In Amritsar Paunda cells forming the parenchymatous matrix were smallest of all the three varieties (Plate IV, Figs. 1, 2, 3 and Figs. 4, 5, 6). The thickness of parenchymatous cell-walls at 1·2 mm. from the epidermis was just a little less than that in the Peshawar Paunda, the difference being insignificant even at 5% level. It is interesting to note that in these two characters also it was significantly different from the Saharanpur variety at even 1% level. The latter exhibited the lowest magnitude (Plate III, Fig. 1) for characters 4, 5, 6 and 7 and was significantly different at 5% level from the variety nearer to it in the three underlined characters and at 1% level in two only namely the 6th and the 7th character. As regards characters 3 and 8, it occupied an intermediate position and was significantly different in respect of the 3rd character only. The ground tissue in this variety was, therefore, composed of largest cells with thinnest walls (Plate IV, Figs. 1, 2, 3 and Figs. 4, 5, 6).

The Peshawar Paunda was situated in between these two varieties so far as characters 4, 5 and 6 were concerned, and was significantly different in the 4th and the 6th characters at 5% and 1% level respectively. In respect of the 3rd and the 8th characters it was least developed but showed maximum development for the 7th character. It was significantly different in the 3rd

TABLE I

Showing various anatomical features of 3 varieties of chewing canes.

Characters \ Varieties	Saharan-pur Paunda	Peshawar Paunda	Amritsar Paunda	General mean	Critical difference at 1%	Critical difference at 5%
Rind: Peripheral Region						
A. *Vascular bundles* :						
(1) Number per unit area ..	9·94	10·25	12·92	11·04	1·33	1·00
(2) Size ..	177·7	147·4	170·9	165·7	*	*
(3) Total area occupied by them in a unit area ..	1767·4	1511·6	2207·8	1828·8	148·5	112·5
B. *Number of Parenchymatous cells* :						
(4) In outer unit area ..	865·2	902·3	1156·3	974·6	43·5	33·1
(5) In inner unit area ..	138·6	140·6	147·1	142·1	8·35	6·35
C. *Thickness of cell-walls* :						
(i) Parenchyma :						
(6) At 0·6 mm. ..	8·00	8·81	9·72	8·84	0·57	0·43
(7) At 1·2 mm. ..	6·81	8·15	8·10	7·69	0·46	0·35
(ii) Sclerenchyma :						
(8) In sheath ..	18·19	17·56	21·95	19·24	1·63	1·24
Storage tissue : Central Region						
A. *Vascular bundles* :						
(9) Number per unit area ..	11·22	11·60	9·22	10·67	1·77	1·30
(10) Size ..	274·6	334·7	401·9	337·1	47·3	36·00
(11) Total area occupied by them in a unit area ..	3089·4	3684·7	3628·2	3455·4	1125·4	830·5
B *Parenchymatous cells* :						
(12) Number per unit area ..	121·8	160·0	142·0	141·3	14·69	10·68
C. *Thickness of cell-walls* :						
(13) In parenchyma ..	6·63	7·73	7·17	7·18	0·84	0·64
(14) In sclerenchyma ..	14·97	14·07	13·23	14·09	1·74	1·32

Note.—1. Since the number of observations for the character (asterisked) was unequal, three pairs of C. D. (critical differences) would be necessary. They are given below:—

Varieties	*At 1%*	*At 5%*
Between Saharanpur and Amritsar Paunda	22·8	17·3
Between Saharanpur and Peshawar Paunda	22·6	17·2
Between Peshawar and Amritsar Paunda	24·0	18·3

2. The magnitude of various features was given in the above table in divisions of eye-piece micrometer, the values of which were:
   (*a*) For thickness of cell-walls:
   (Characters 6, 7, 8, 13 and 14) 1 division = 0·21 μ.
   (*b*) For area of vascular bundles:
   (Characters 2, 3, 10 and 11) 1 sq. division = $16 \cdot 3^2$ sq. μ.

3. (*a*) For counting the number of vascular bundles, the field of the microscope was taken as unit area, the diameter of which was as follows:
   (i) Character 1 : 1·7 mm.
   (ii) Character 9 : 4·1 mm.

   (*b*) For the number of parenchymatous cells, the diameter of the field of microscope was taken as one of the sides of the square which measured:
   (i) 0·68 mm. for characters 4 and 5.
   (ii) 1·53 mm. for character 12.

character only at 1% level. The Peshawar Paunda on the whole, therefore, occupied an intermediate position so far as the rind was concerned.

The storage tissue.—In the central region, however, the Peshawar Paunda showed maximum magnitude for the 11th, 12th and 13th character and was intermediate for the 14th. It formed groups with the Saharanpur or Amritsar varieties or with both in all the characters except the 12th (Plate I, Figs. 4–6) where it was significantly different from both of them even at 1% level, the difference in the size of the parenchymatous cells being clearly visible (Plate IV, Figs. 7, 8, 9). The Saharanpur Paunda, in this region also, was the poorest in the development of characters 11, 12 and 13 (Plate III, Fig. 4). Only in the case of the 14th character it showed the highest value. In the 12th character alone it was significantly different from the other two varieties at 1% level. In other words, the storage cells were the largest in this case (Plate IV, Fig. 8). At 5% level it differed significantly from Amritsar Paunda in the 14th character, and from the Peshawar Paunda in the 13th character. The values for 11th, 12th and 13th characters for the Amritsar variety were intermediate and were not significantly different except for that of the 12th character (Plate III, Fig. 6). Its average value for the 14th character was the smallest and significantly different from the one for the Saharanpur Paunda at 5% level.

Summing up all the characters exhibited by the three varieties, Amritsar Paunda headed the list for five out of the ten characters and was significantly different in all of them. It was intermediate in four characters but differences in respect of three of them were not significant as compared to their maximum development. Only in one character it showed the least development but here too, the difference between this and the variety intermediate for this character (Table I) was not significant. Amritsar Paunda, therefore, could be deemed to have secured eight top positions and two middle ones. For the Saharanpur Paunda, reverse was the case. It showed the least development for seven characters of which four were significantly different at 5% level. It had intermediate position for two characters but the difference between this and the lowest was significant only for one, at 5% level. In one character alone, it topped the list but it was not significantly different from the next variety. Thus it obtained for itself eight lowest and two intermediate positions. The Peshawar Paunda was intermediate as it had one distinctly highest and one definitely lowest position, the other eight being intermediate. The position of a variety in relation to the other two in respect of characters in which it was found to be significantly different is shown in Table II.

TABLE II

Showing characters in which varieties are significantly different from one another

Characters \ Varieties	Saharanpur Paunda	Peshawar Paunda	Remarks
Rind :			
Peshawar Paunda ..	4, 6, 7 / 3	..	Amritsar Paunda is the hardest of all in rind.
Amritsar Paunda ..	3, 4 5, 6, 7, 8	3, 4, *5*, 6, 8	
Storage tissue :	..	..	
Peshawar Paunda ..	12, 13 / ..	..	Peshawar Paunda is the hardest of all in core.
Amritsar Paunda ..	12 / *14*	.. / 12	

Note.—1. Differences in characters italicized are significant at 5% level only, the rest at 1% level.

2. Characters given above the horizontal line were better developed in the variety given on the left than in that given at the head of the column. Reverse was the case when a character appeared below the horizontal line.

The full import of this consistent behaviour on the part of the varieties would be clear when all the characters were considered together.

IV. Discussion of Results

A cane when cut across, reveals two component parts, *viz.*, the rind and the core. The rind is formed by vascular bundles of various sizes, embedded in a parenchymatous matrix lignified to a varying degree. The character of the rind is, therefore, determined by the total area of vascular bundles, the size of parenchymatous cells and the thickness of cell-walls in both the tissues. The function that rind has to perform, naturally admits of little storage of sugar in its tissues. The core which forms the major portion of a cane, consists of thin-walled parenchymatous tissue in which isolated vascular bundles are scattered rather sparsely.

In a chewing cane, the rind has got to be differently constituted from that of a cane for general cultivation (*vide* Appendix). A soft rind for the latter is definitely a bad feature because of its inability to withstand the ravages of smaller animals like rodents and jackals, while in the former it is a valuable asset as the human jaw and teeth are comparatively a weak mechanism for biting and tearing purposes. At the same time it should not be so soft as to come off in chips when the cane is peeled. It should, therefore, be soft but coherent enough to be stripped from node to node. Besides, if it is thin and well demarcated also, the cane would be ideal so

far as one part of chewing process is concerned. A soft rinded cane need not present any serious obstacle in successful cultivation, because it being a garden crop, damage by animals can be reduced to a great extent by growing them in properly protected plots.

The core of a chewing cane is not fundamently different from that of an ordinary one, meant for crushing (*vide* Appendix). Only it has to be much softer so that it can be pressed by human jaw and juice extracted from individual cells. It is, therefore, essential that the storage cells should be as large as possible with very thin walls. The vascular bundles should obviously be spaced far apart.

From these theoretical considerations, a cane possessing a thin and soft rind and yet adequately tough to be stripped clean from node to node, together with a soft core requiring the least effort to peel and crush it, would be ideally suited for chewing purpose.

Before discussing the relative merits of the three varieties as chewing canes, it would be necessary to eliminate the inequalities of numerical expression of various characters, so that their magnitudes could be compared to one another. Table III was compiled by expressing the magnitude

TABLE III

Showing the magnitude of various characters as percentages of their respective totals

Characters \ Varieties	Saharanpur Paunda	Peshawar Paunda	Amritsar Paunda
Rind—Peripheral region—			
A. Vascular bundles :			
(1) Number per unit area ..	30·0	31·0	39·0
(2) Size ..	35·8	29·7	34·5
(3) Total area occupied by them in unit area ..	32·2	27·5	40·2
B. *Number of Parenchymatous cells* :			
(4) In outer unit area ..	29·6	30·9	39·6
(5) In inner unit area ..	32·5	33·0	34·5
C. *Thickness of cell-walls* :			
(i) Parenchyma (6) At 0·6 mm. ..	30·2	33·2	36·6
(7) At 1·2 mm. ..	29·5	35·3	35·1
(ii) Sclerenchyma (8) In sheath ..	31·3	30·4	38·0
Storage tissue—Central region :			
A. Vascular bundles :			
(9) Number per unit area ..	35·0	36·2	28·8
(10) Size ..	27·2	33·1	39·7
(11) Total area occupied by them in a unit area ..	29·7	35·4	34·9
B. *Number of Parenchymatous cells* :			
(12) In unit area ..	28·7	37·8	33·5
C. *Thickness of cell-walls* :			
(13) In parenchyma ..	30·8	35·9	33·3
(14) In sclerenchyma ..	35·4	33·3	31·3

of a character in a particular variety as percentage of its total for all the three varieties.

From Table II given earlier, it would appear that even at 1% level, the Amritsar variety was significantly different from the Saharanpur Paunda in all the six characters of the rind and from the Peshawar Paunda in four of them. Further it maintained consistently the highest position (Text-Fig. 1) in respect of the magnitude of almost all characters each of which made the rind hard to the extent of its development. It was, therefore, evident that the Amritsar variety had the hardest rind. In respect of the storage tissue, the Peshawar Paunda was harder than the Saharanpur variety, because the number and thickness of walls of the parenchymatous cells—two out of four characters pertaining to the core—were significantly greater in the former than in the latter. Although the lignification of sclerenchyma was higher in the Saharanpur variety than in the Peshawar Paunda, the difference between the two magnitudes was not found to be significant,

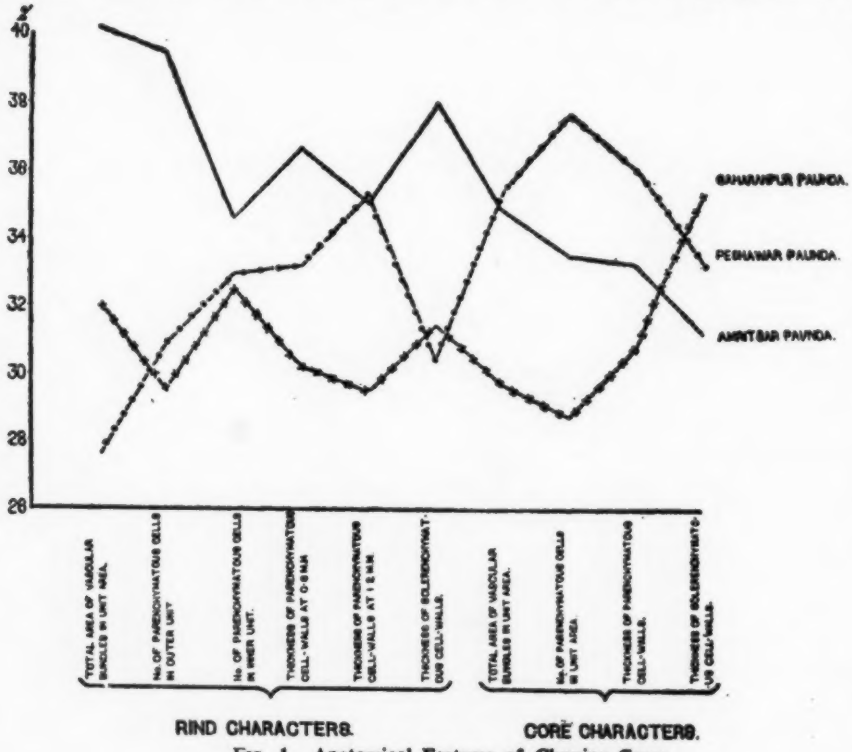


FIG. 1. Anatomical Features of Chewing Canes.

and as such greater thickness of cell-walls in the former could not be regarded as having materially contributed much to make its core harder than that of the latter. As compared to the Amritsar variety also, the core of the Peshawar Paunda appeared to be hard because of the significantly greater number and therefore smaller size of parenchymatous cells per unit area.

Since the Saharanpur Paunda changed positions with Peshawar variety in respect of certain characters in rind and with the Amritsar variety in those of the core, it might not be the softest because it was just possible that the greater development of some of the characters might more than offset the total advantage given by the poorer development of the rest of the features in their respective spheres. If the characters in which they were not found to be significantly different, were left out in the final reckoning of hardness for the reason stated in the preceding para, one had only to examine whether hardness imparted to the rind by greater area of vascular bundles in the Saharanpur variety was so much as to nullify the total softness due to the poorer lignification of the parenchymatous matrix as revealed by the thickness of cell-walls at depths of 0·6 mm. and 1·2 mm. from the epidermis and the lesser number and therefore larger size of parenchymatous cells in the outer unit area. Similarly for the core, it would be necessary to find out whether the greater development of sclerenchyma had more than counter-balanced the effect of lesser number and therefore the larger size of the parenchymatous cells per unit area. A similar reckoning of hardness of the regions in question for the other two varieties, *viz.*, Amritsar and Peshawar would be essential before any comparison could be made.

In assessing the combined effect of different characters which pulled in opposite directions, weightage given to any one of them on theoretical considerations would be more or less arbitrary as no direct experimental evidence could be obtained on this point. Since the total area of vascular bundles and the thickness of parenchymatous cell-walls are distinctively varietal characters and independent of each other, they have to be taken as of equal status. Whether the magnitude of the lignification of parenchymatous cells at each depth in the rind is independent of the other and, therefore, should get the same weightage as the vascular area, or each is the function of the other and therefore, the two being interdependent, can be best represented on 50–50 basis, has to be decided before evaluating the total hardness of the peripheral region in a cane.

If the thickness of parenchymatous cell-walls at 1·2 mm. had shown the same ratio to that at 0·6 mm. in all the three varieties, it could not have

been explained except on the basis of the former being dependent on the latter. But that is not so (Text-Fig. 2). That the lignification of cell-walls

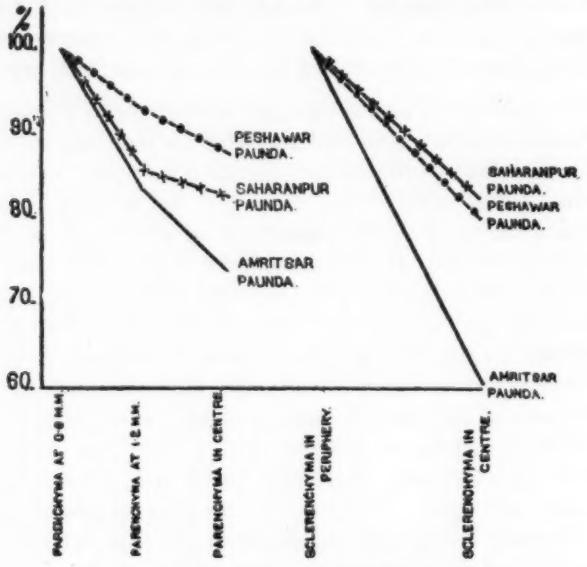


FIG. 2. Thickness of Cell-Walls.

in different parts of the cross-section of a cane was an independent character was further supported by the fact that in all the three varieties the thickness of parenchymatous cell-walls at 1·2 mm. did not show the same relationship to that in the centre, and that the rate of its fall also was not the same for all. Observations on sclerenchyma confirmed this view (Text-Fig. 2).

On this basis, giving equal weightage to all the three characters, the Saharanpur Paunda was found to be four points less hard than Peshawar Paunda at 1% level (Table IV). At 5% level it gained one more point because of the lesser number of parenchymatous cells per unit area. Even if characters 6 and 7 were taken as components of one feature and not at par with the third character, each therefore, being eligible to only 50% weightage of the latter, the Saharanpur variety appeared to be just 0·3 point harder than the Peshawar Paunda at 1% level and one point less hard at 5% level because of the fourth character coming into the picture. So in 3 out of 4 computations the former scored over the latter as being less hard so far as the rind was concerned. In the core also, it was slightly (0·7 point) superior to the Amritsar cane at 5% and considerably (4·8) so at 1% level.

TABLE IV

Showing weightage of characters in which Saharanpur Paunda is significantly different from other varieties

Characters	Saharanpur Paunda	Peshawar Paunda	Saharanpur Paunda (+) or (−)	Remarks
(a) *In Rind*:				
At 1% level:				
$3 + 6 + 7$ ..	91·9	96·0	−4·1	Saharanpur is softer than Peshawar Paunda
$3 + \frac{6+7}{2}$ ..	62·0	61·0	+0·3	More or less equal
At 5% level:				
$3 + 4 + 6 + 7$ ..	121·5	126·9	−5·4	Saharanpur Paunda is softer than Peshawar Paunda
$3 + 4 + \frac{6+7}{2}$ ..	91·6	92·6	−1·0	
		Amritsar Paunda		
(b) *In storage tissue*				
At 1% level:				
$12 + 14$ ..	64·1	64·8	−0·7	Saharanpur Paunda is softer than Amritsar Paunda
At 5% level:				
12 ..	28·7	33·5	−4·8	

Note.—Since greater magnitude means more of hardness, (+) indicates "harder than" and (−) "softer than".

In addition to its being the softest of all the three varieties, Saharanpur Paunda had another advantage. Due to the rapid fall in the lignification of the matrix, its rind was thinner and much better demarcated than that of the other two. In this case there was practically no further decrease in the magnitude of this feature (Text-Fig. 3), as one approached the storage tissue whereas in the other two varieties, such delineation was not found to exist, within 1·2 mm. from the epidermis. Should it occur farther in, the Saharanpur Paunda would at least retain the desirable feature of having a thin rind, which comes off easily and clean when stripped. The rind of the other two varieties due to greater and uniform thickening of the cell-walls of the matrix would obviously require more effort to peel.

From the foregoing discussion it would appear that of the three varieties, the Saharanpur Paunda was found to be the closest to the ideal chewing cane both in respect of the rind and the storage tissue. The Amritsar Paunda came next in respect of the latter region but was the farthest so far as the rind was concerned. The Peshawar variety occupied an intermediate position as regards the rind but was the hardest in the core.

It is interesting to note that the shape of parenchymatous cells was different in these varieties (Plate IV, Figs. 1–9). They were strongly oval

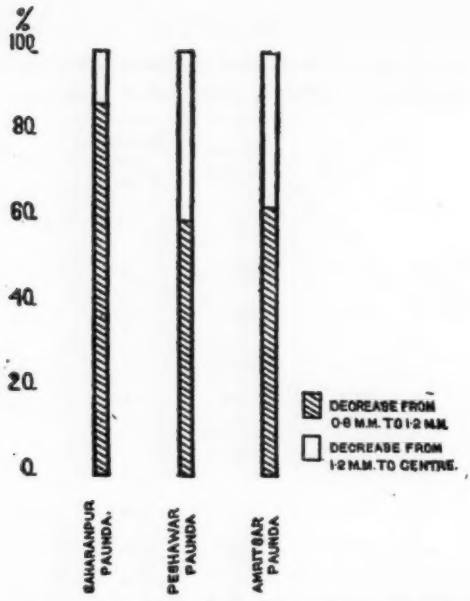


FIG. 3. Decrease in the Thickness of Parenchymatous Cell-Walls.

in the case of Amritsar Paunda, and less so in that of Saharanpur var iety while in the Peshawar Paunda they were almost circular. The shape of these cells, however, did not appear to have any bearing on the suitability of a variety as a chewing cane.

V. SUMMARY

1. The present paper tries to visualise from anatomical point of view an ideal chewing cane which besides having a soft core, possesses a thin and well-defined rind that comes off clean with minimum effort when it is peeled.

2. The following characters which imparted hardness to the core and rind of the cane stalk were studied for three well known chewing-canes, *viz.*, the Amritsar Paunda, the Saharanpur Paunda and the Peshawar Paunda.

(*a*) Total area under vascular bundles per unit area as calculated by adding the products of the radial and targential axes of all the vascular bundles found in the field of the microscope. The product of the two axes of a vascular bundle in peripheral region bore a very high positive correlation of the order of 0·9546 with its area as determined by a planimeter.

(*b*) The number of parenchymatous cells per unit area, which obviously was inversely proportional to their size.

(*c*) The thickness of cell-walls in the parenchyma at two depths from the epidermis, *viz.*, 0·6 mm., 1·2 mm. and in centre, and in the sclerenchyma, in periphery and centre.

3. The Amritsar and Peshawar varieties were hardest in the rind and the core respectively because of the maximum development of all the characters in those regions.

4. The rind of the Saharanpur variety was softer than that of the Peshawar Paunda, because the poorer lignification of the parenchymatous matrix in the former more than compensated for its greater area under vascular bundles whereas in the latter variety, the ground tissue was lignified so highly that it offset total softness accruing from lesser area of vascular bundles. Moreover, due to the rapid decrease in the thickness of cell-walls of the ground tissue, the rind was thin and well demarcated so that it peeled off clean with minimum effort.

5. In the core, the Saharanpur Paunda had the smallest number of storage cells per unit area and, therefore, the largest in size, on the basis of which it was reckoned as softer than the Amritsar Paunda because in other characters, *viz.*, the vascular area, the lignification of the parenchyma and sclerenchyma, it was not significantly different from that variety at 5% level.

6. The Saharanpur Paunda was, therefore, closest to the ideal chewing cane and the respective positions of the three varieties might be graphically shown as below:

Chewing cane { Rind—the Saharanpur—the Peshawar—the Amritsar.
Core—the Saharanpur—the Amritsar—the Peshawar.

7. A chewing cane was found to be constituted on a pattern entirely different from that of a factory cane. The vascular bundles in the rind region of these varieties of chewing canes were found to be nearly half the size of those of the factory canes of the province. The total area under them in unit area was much higher in the latter than in the former. The cell-walls in both the tissues all over the cross-section were found to be much more highly lignified in the factory canes than in the chewing varieties.

VI. Acknowledgements

The work was carried out at the Central Sugarcane Research Station, being jointly financed by the Bihar Government and the Indian Central Sugarcane Committee to whom grateful thanks are due. The assistance

rendered by Mr. K. S. Bandopadhay, Statistician at the Station, in analysing the data is also acknowledged.

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Explanation of Plates

Plate III

T. S. of Stem of Chewing varieties of Sugarcane showing the Rind and Storage Tissues

Rind Tissue. Figs. 1–3. Fig. 1. Part of T. S. of rind of Saharanpur Paunda showing parenchymatous matrix formed by cells larger than those of the Amritsar variety (Fig. 2), but with poorer lignification. The rind of the latter variety has greater number of vascular bundles than those of the Saharanpur and Peshawar Paundas (Figs. 1 and 3); and so also the thickening of sclerenchymatous cell-walls resulting in great reduction of the lumen of cells. (Magnification × 80.)

Storage Tissue. Figs. 4–6. Fig. 4. Part of T. S. of the storage tissue of Saharanpur Paunda showing cells larger in size than those of the Peshawar Paunda (Fig. 6). The Amritsar variety (Fig. 5) occupies an intermediate position in this respect but has the smallest number of vascular bundles. (Magnification × 15.)

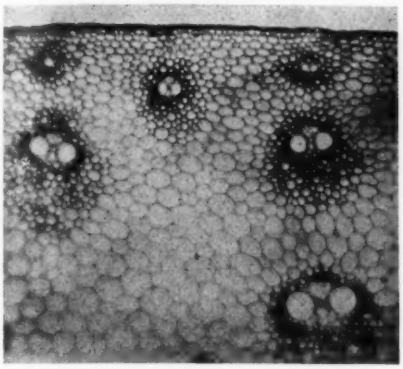
Plate IV

T. S. of Stem showing the Size of Parenchymatous Cells at Three Depths from the Epidermis

Figs. 1, 2 and 3 at 0·6 mm., Figs. 4, 5 and 6 at 1·2 mm. and Figs. 7, 8 and 9 at 2·4 mm. In Amritsar Paunda (Figs. 1, 4) the cells forming the parenchymatous matrix of rind are the smallest and most highly lignified, whereas in the Saharanpur variety (Figs. 2, 5) they are the largest in size with thinnest walls. The Peshawar Paunda (Figs. 3 & 6) occupies an intermediate position being nearest to the Saharanpur Paunda.

In the storage tissue, however, the former has the smallest cells (Fig. 9) followed by the Amritsar and Saharanpur varieties (Figs. 7 and 8). The shape of the cells in the three varieties is also different. (Magnification × 200.)

RIND TISSUE



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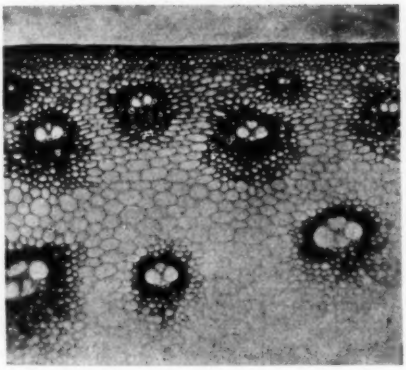

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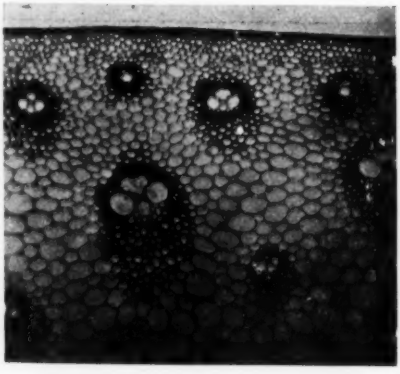

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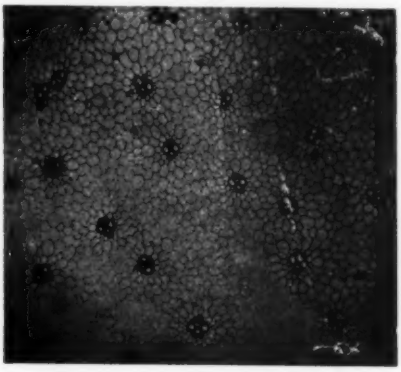
STORAGE TISSUE



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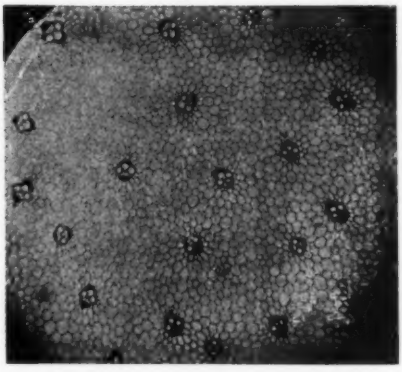

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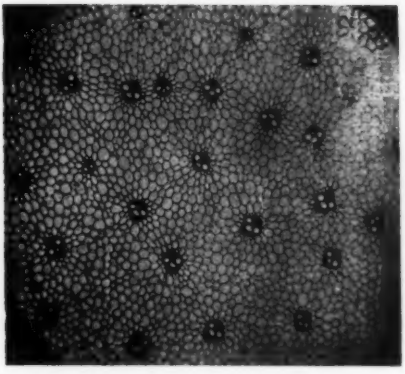

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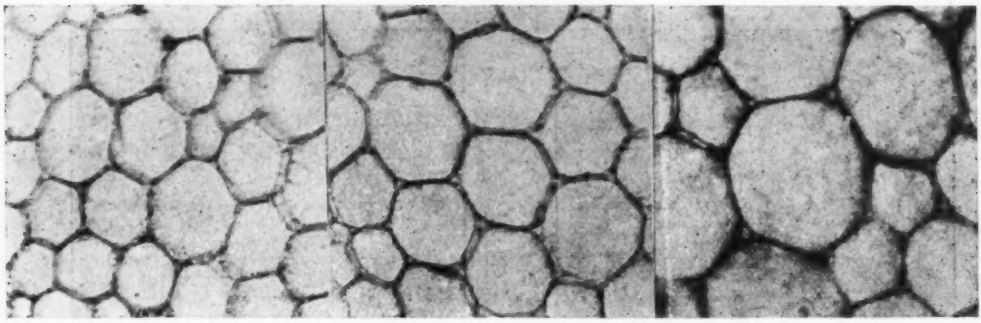

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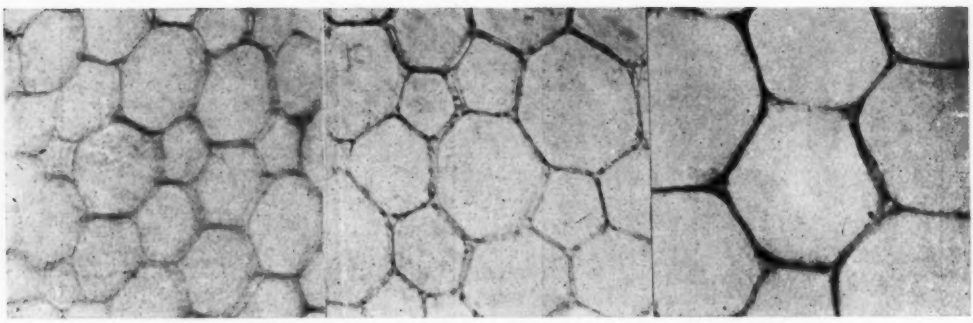

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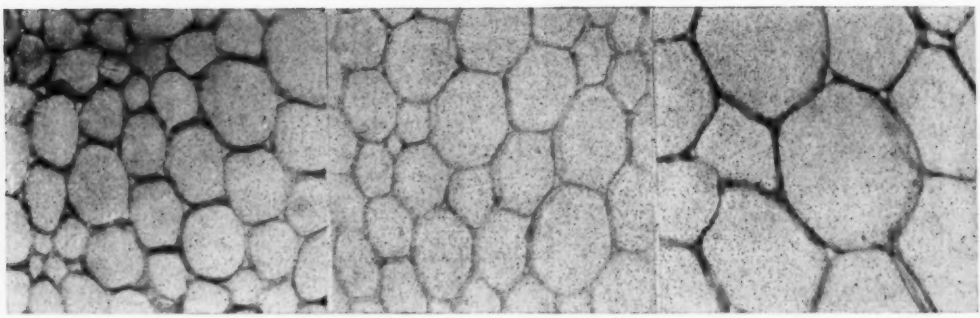

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APPENDIX

Characteristic Features of a Factory Cane

A factory cane is characterised by a comparatively high fibre content, as distinct from that used for chewing purposes. A low fibred variety apart from presenting considerable mechanical difficulties in crushing, would not be an economical proposition to mill because of the insufficient bagasse which is used as a fuel. Moreover, it should be hardy enough to withstand the vagaries of weather and other adverse circumstances so that it can be grown on an area commensurate with the needs of a factory.

These fundamental differences between the two categories of cane varieties are broad-based on their anatomical make-up (Table I). In the case of a chewing cane, the rind is soft and lignification of tissues, both parenchymatous and sclerenchymatous all over the cross-section of a cane, poor, while a factory cane has to possess a fairly hard rind and moderately lignified tissues.

While in the rind the number of vascular bundles per unit area in the factory canes was significantly less than that in the chewing varieties except the Saharanpur Paunda, their size and the total area occupied by them in the former group were nearly twice as much as were found in the chewing canes. In the case of the storage tissue although the number of and total area occupied by vascular bundles were not directly comparable because of the difference in the size of unit area taken for the two types of cane varieties, there did not appear to be any appreciable difference between the two groups of the cane varieties so far as these characters were concerned. This view appeared to be supported by the average size of individual vascular bundles which was more or less the same in the two groups, because the difference in its size in various varieties was not found to be significant even at 1% level.

The difference in the anatomical make-up was much more categorical when the thickness of cell-walls of the parenchymatous and sclerenchymatous tissues, was considered. In Co 210 and Co 213 the thickness of the cell-walls in the parenchymatous matrix at both the depths in the peripheral region was nearly three times and in the central region approximately twice of the general mean for the chewing canes. As regards the sclerenchyma forming the vascular sheaths in the rind, the lignification of cell-walls in Co 210 and Co 213 was nearly three and two times respectively of that found in the chewing varieties. In the central region, however, the difference as regards this feature was not so pronounced. Still the thickness of these cell-walls was nearly 25% as much more in these varieties

TABLE I

Showing anatomical features of factory and chewing sugarcane varieties

Character	Chewing canes				Factory canes			Critical diff. bet. the two groups at 1% level
	Amritsar	Saharanpur	Peshawar	General mean	Co 210	Co 213	Co 313	
Rind : Peripheral region—								
A. Vascular bundles :								
(1) Number per unit area	12·93	9·94	10·25	11·04	9·17	8·65	8·96	1·028
(2) Size	170·96	177·73	147·41	165·7	405·50	316·37	367·66	17·970
(3) Total area occupied by them in unit area ..	2207·8	1767·4	1511·0	1828·8	3708	2734	3295	..
B. *Thickness of cell-walls* :								
(4) Parenchyma at 0·6 mm.	9·78	8·00	8·81	8·84	23·13	23·31	11·56	0·984
(5) Parenchyma at 1·2 mm.	8·10	6·81	8·15	7·69	23·69	21·81	11·56	1·151
(6) Sclerenchyma ..	21·96	18·19	17·56	19·24	54·50	39·31	29·94	2·396
Storage tissue : Central Region—								
A. *Vascular bundle—*								
(7) Number per unit area ..	9·22	11·22	11·60	10·67	3·44	3·73	3·14	..
(8) Size ..	401·87	274·61	334·74	337·1	359·59	306·17	362·63	46·597
(9) Total area occupied by them per unit area ..	3628·2	3089·4	3684·7	3455·4	1237	1142	1139	..
B. *Thickness of cell-walls—*								
(10) Parenchyma ..	7·17	6·63	7·75	7·18	13·94	13·56	13·44	1·375
(11) Sclerenchyma ..	13·23	14·96	14·08	14·9	18·36	17·50	21·19	2·320

Note.—1. The magnitude of various characters was expressed in terms of the divisions of the eye-piece micrometer the values of which were:

(*a*) Thickness of cell-walls : 1 division = 0·21 μ.

(*b*) Size and total area of vascular bundles in

(i) rind 1 sq. division = $14 \cdot 4^2$ sq. μ For factory canes.

(ii) Storage tissue 1 sq. ,, = $15 \cdot 2^2$ sq. μ ,,

(iii) both the regions 1 sq. ,, = $16 \cdot 3^2$ sq. μ For chewing canes.

2. For counting the number of vascular bundles the field of the microscope was taken as unit area the diameter of which was as follows :

(i) 1·7 mm. for both the regions of factory canes and rind portion of the chewing varieties.

(ii) 4·1 mm. for the central portion of the latter.

3. It would therefore appear that the magnitude of the features under discussion were more or less directly comparable in both the types of cane varieties except the number of and total area occupied by vascular bundles per unit area in the storage tissue where the diameters of unit area happened to be different.

4. Critical difference for characters 7 and 9 could not be calculated for the reason given above, and for the character 3 because the total area was arrived at by two different methods.

than that found in Paunda varieties. Co 313 which was the softest of all the varieties given out so far, for general cultivation as a factory cane, was found to have nearly 50% more highly lignified cell-walls in both the tissues all over the cross-section. It was harder than the hardest of the chewing

varieties, namely the Amritsar Paunda. All the three varieties were significantly different from the chewing canes at 1% level in respect of the lignification of both the tissues.

It would therefore appear that cane varieties in order to be useful for different purposes have got to be constituted on an entirely different basis. The work on milling features of sugarcane varieties is being reported separately.

THE TAPIOCA PLANT AND METHODS FOR EVOLVING IMPROVED STRAINS FOR CULTIVATION*

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I. Introduction

Tapioca or the Cassava plant (*Manihot utilissima*, Pohl.) belongs to the milkweed family (Euphorbiaceæ). It is a native of Brazil, South America. In the botanical literature of the last century the plant is described as *Janipha manihot*, Kth. or *Jatropha manihot*, Linn. It was introduced in this country more than a century ago. Drury in his " Useful Plants of India" published in 1858 has recorded that tapioca was then under extensive cultivation in Travancore. The Travancore State Manual has

* A Monogram on the work done in the Department of Research, University of Travancore, during 1940–47.

stated ' that the popularity of this crop plant is specially due to the exertions of His Highness Sri Visakham Thirunal Maharaja ' (1837–1885). Burkill (1904) states that early Portuguese settlers introduced tapioca to Goa as well as to Africa. Macmillan (1925) records that Tapioca was introduced into Ceylon and India by the Portuguese in the 17th century.

The success of Tapioca as a major crop has in large measure been due to the particularly favourable climatic and soil conditions of Travancore. It is also likely that the growth habit and high yield of this plant ensured its popularity with the ryot. It is a hardy plant thriving even in the most barren soil. Little or no care is necessary for it after planting and the yield per acre is so high as to give for the ryot a good return for his labours. In pre-war days a pound of fresh tapioca tubers did not cost more than two pies. It was thus within easy reach of the poor and became practically the poor man's food in Travancore. Tapioca cultivation in consequence steadily increased so that to-day it is second only to paddy as a major crop in this State.

Owing however, to the primitive methods of cultivation employed by the ryot and the poor quality of the varieties used for cultivation, the average yield here does not exceed two tons of tubers per acre at present. In Java, West Indies and other countries of the Far East where improved strains of tapioca are used for cultivation, the average recorded yield for this crop ranges from 10–15 tons per acre. It should therefore be possible substantially to increase the yield of this crop by enabling the ryot to have better varieties of tapioca and by introducing improved methods of cultivation.

During the war when rice imports from Burma were cut off, tapioca has been a boon to Travancore. It saved the country from famine and its food value has received greater attention since then. Tapioca has also assumed importance in recent years as its starch is in great demand as a suitable sizing material in textile industry. There is no doubt, therefore, that the cultivation of this crop will receive greater attention in future. The following account of the applications of modern genetical methods for evolving improved strains of tapioca undertaken at the Tapioca Research Farm* in the Department of Research, University of Travancore, is therefore presented with a view to stimulating interest in this crop plant.

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* This Farm is maintained from the Pattabhirama Iyer Endowment Funds donated at the rate of Rs. 1,000 per mensem by Sachivottama Sir C. P. Ramaswamy Iyer, Dewan Vice-Chancellor.

II. The Tapioca Plant

Tapioca, known as '*maracheeni*' or '*kappacheeni*' in Malayalam is a crop plant cultivated in all dry soils in Travancore. Within a week after planting, two or three buds sprout from the nodes of the seed canes and grow up as erect branches. These stems branch repeatedly in threes and run into several such grades in an apparently trichotomous mode of branching (Fig. 1). While this type of branching with a spreadings shoot

Fig. 1. The Tapioca Plant

system is characteristic of all flowering varieties, it is significant that non-flowering varieties seldom branch, growing as erect, tall plants, reaching a height of 6–8 feet. The colour of the stem varies with varieties. It may be green, grey, pink, dark-brown or purple. Leaves stipulate, long petioled, palmate, divided nearly to their base into 5–7 lanceolate, entire lobes dark green above and glaucous beneath. Midrib prominent below and usually of the same colour as the petiole. Petiole long, inserted obliquely on the stem and arranged in a 2/5 spiral. Stipules thin, dissected, pointed, greenish white, occasionally with a reddish base falling off just after the leaf has fully spread. On an average about 15 leaves will be present on the terminal region of the branch. Leaves become mature and fall within about six weeks, leaving prominent nodal protuberances on the stem. These nodal swellings are surmounted by circular leaf-scars with an obliquely transverse knife-edge like extension on each side formed by the stipular scars. The stem thus presents a rugged exterior with these close-set and spirally arranged swellings. Cork formation commences early on the stem developing a scaly skin which can be easily peeled off.

Roots grow from the cut-end of the seed cane within a few days after planting. They are long, slender and white, spreading in the soil more or less horizontally about 3–5 inches below the surface. As the plant grows

FIG. 2. Young Plant growing from a cutting

older, some of these roots become tuberous while the majority of them continue to be thin and function as absorbing organs. Both absorbing and tuberous roots are spreading in habit so that hardly any root lies deeper than 8–10 inches in the soil. As the tuberous roots begin to store starch in them they gradually increase in thickness, developing when mature, a skin as in the stem, a rind and the starchy inner portion with a central strand of conducting tissue. In most varieties growth of the tubers is completed in about eight months.

The plants flower in about six months. The flowers are borne in terminal panicles. The first panicles usually appear at the junction of branches of the second grade (Fig. 3). Flowers unisexual, protogynous.

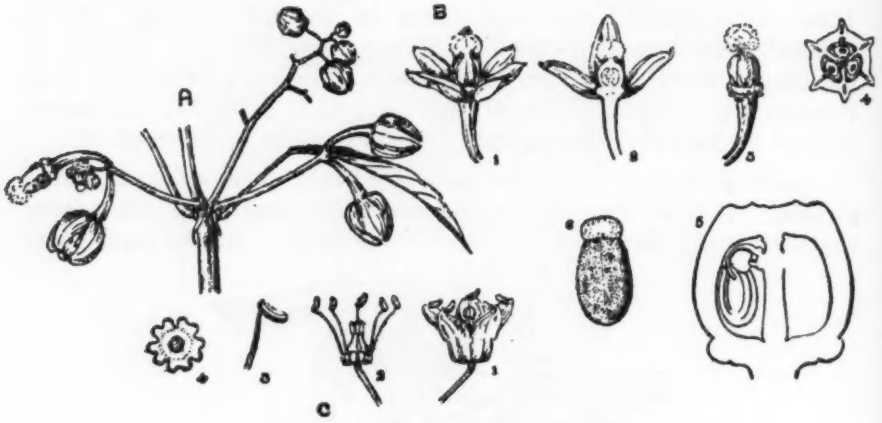

FIG. 3. Flowers and Fruit of Tapioca

Male flowers smaller than the female and usually in terminal clusters. Perianth cup-shaped, with five imbrecate lobes enclosing a 10-lobed glandular disc. Stamens 10, springing from the base of the perianth and curving out through the lobes of the disc. In the open flower the anthers are arranged in two levels; five small with shorter filaments curved inwards and five large with spreading filaments. The small stamens are opposite the perianth lobes while the large ones alternate these lobes. Anthers dorsifixed and dehisce by longitudinal slits. Pollen grains are large, spherical and few in each sac. In the centre of the disc a pistillode is represented by a small conical projection. The female flowers are developed earlier than the male flowers of the panicle and are fewer in number. The perianth of the female flower is deeply 5-lobed and with purple border. Pistil has a basal ring. Ovary trilocular, spherical with a capitate 3-lobed

stigma. Ovule one in each loculus, pendulous, anatropous with ventral raphe and micropyle directed upwards and outwards. At the tip of the outer integument a soft tissue, the caruncle, is formed which caps the ovule. There is also an obturator mechanism which is a peg-like process formed from the placenta. This grows towards the ovule, curves round the caruncle and enters the nucellus through the micropyle (Fig. 3, B. 5). It is composed of thin-walled, elongated cells with rich contents. The pollen-tube in its passage to the embryosac directs its course through the obturator. This interesting mechanism thus serves as a short-cut to the micropyle besides being a nutritive tissue for the pollen tube as suggested by Strasburger (1921). After fertilisation the obturator disappears.

Fruit globular, about one inch in diameter with six thin, narrow wings. Capsule hard, splitting into three cocci each with a seed. Seeds elliptical, black, grey or mottled, shining, resembling a castor seed. The seed-coat is thick, hard and polished. Inside the seed-coat there is the endosperm which is massive enclosing the embryo. The two leaf-like cotyledons of the embryo are pressed against each other by the endosperm. The radicle of the primary axis is directed towards the micropyle.

During germination the radicle pushes itself out through the micropyle and develops branch roots at its tip. The hypocotyl is curved and by its further elongation the cotyledons are pulled out of the seed (Fig. 4). They then expand, become green and behave like foliage leaves.

Anatomy of the Stem

(*a*) *Macroscopical.*—The stem is light, breaks easily and has a thin skin which gets easily peeled off exposing a green soft tissue inside. A cut-end of the stem shows four prominent parts—a central whitish pith, a broad ring of wood, a greenish bast and a brown skin. The pith is a soft tissue about a centimeter in diameter in a young stem of normal thickness. It is pentangular with rounded corners. This tissue is enveloped by the wood. The radial arrangement of the cells of the wood are well marked out on the cut surface. Pores though few are conspicuous and regularly arranged. Milky latex with a faint yellow colour is seen to exude in a ring in the bast and in small drops at the protoxylem region on the cut-end of a fresh stem. The skin is dry and papery. It breaks off in narrow flakes and consequently the surface of the stem is rarely smooth. Lenticels are few. The rind as well as the skin can be easily removed from the stem (Fig. 5).

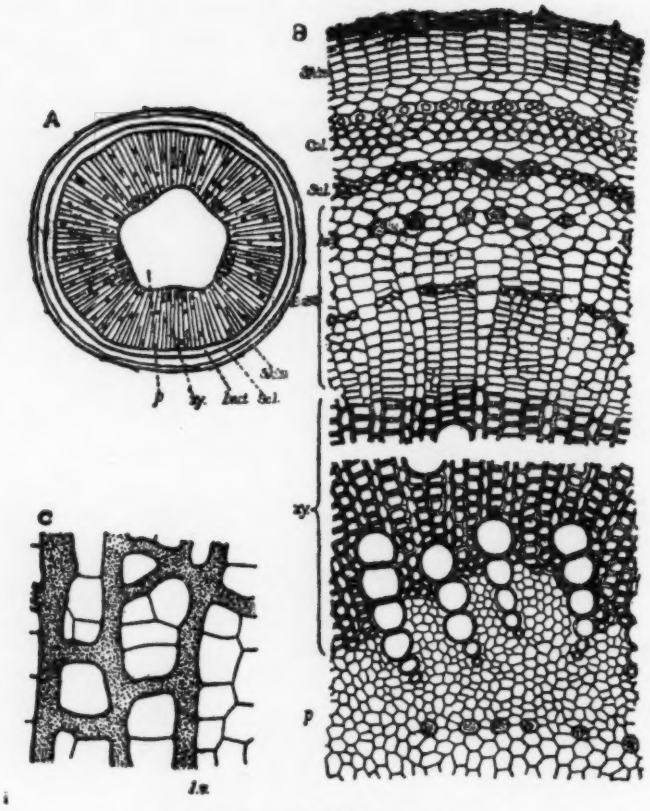

FIG. 5. Cross-Section of Stem

A. Cut end of the stem seen under a lens. B. Tissues seen under the microscope. C. Latex vessels.

(*b*) *Microscopical.*—The skin is the cork tissue developed by a phellogen originating near the epidermis in young stems. It is composed of 8–10 layers of regularly arranged prismatic cells (Fig. 5, B). More than half of these layers on the outside develop thick walls in their cells while the inner layers usually remain thin-walled. The phellogen continues to be active producing newer layers of cork cells as older peripheral layers get peeled off in thin flakes. The cells of the cortex are more or less rounded in outline and have chloroplatids in them presenting a green colour to this tissue. Two or three layers on the inside of the cortex are transformed into collenchyma (Fig. 5, '*col.*'). There is a layer of cells with inclusions of tetrahedral crystals just on the outside of this tissue. Cortex is limited n the inside by a ring of sclerenchyma of two or three layers of thick-

o

walled cells (Fig. 5, '*scl.*'). The fibrous nature of the rind is due to this ring. The tissue inside this ring is the bast. In shape as well as arrangement of cells the bast presents a complicated structure. The inner layers of this tissue have radially arranged cells developed by the cambium. The primary phloem is seen as disorganised groups of cells (usually five) in the outer region. Latex vessels (*l.v.* in Fig. 5) are present just on the border of the secondary phloem. These are branching and anatomosing tubes, extending vertically and arranged only in one layer (Fig. 5, C). The wall of the latex vessel is thin and its contents granular. Between the bast and wood is the delicate ring of cambium. It is composed of thin-walled cells and have their broad side along the tangential plane. The bulk of the wood (Fig. 5, B '*xy.*') is composed of fibres and medullary rays with scanty development of vessels. The medullary ray cells are thin-walled, radially elongated and are rich in starch grains. There may be two or only one such row of cells constituting a ray. These rays become broader as they pass through the bast. The fibres are only moderately thick walled with the result that the wood is soft and light. The vessels though few in number have wide lumen and are conspicuous in the wood. The primary xylem, usually five in number and characterised by radial series of large vessels are seen projecting into the pith at the inner extremity of the wood. Pith is a soft tissue of thin-walled polyhedral cells (Fig. 5, *p*). The outer layers of it are smaller and their walls thicker than those of the central tissue. These cells are also seen to store starch in them. A few latex vessels are developed in this tissue just on the inside of the proloxylem groups. The cells towards the centre are large, polyhedral and thin-walled and have little or no starch grains in them.

Anatomy of the Tubers

The anatomy of the tuber is essentially similar to that of the stem except for the structure of the central vascular strand and of the 'flesh'. The young root has the typical dicotyledonous structure with 4–6 exarch, radial bundles. Endodermis is well developed. Tuber development in it is initiated by the cambium ring. This ring produces towards its inside, almost exclusively, thin-walled cells which act as store houses for starch grains. While these cells have thus the same origin as wood cells following more or less the same arrangement as well, thick-walled fibres are seldom developed with the result that the soft 'flesh' of the tuber is formed. This tissue is thus secondary xylem specially formed for storage. As in the stem it is enveloped by a rind, with almost similar structure. The skin which envelopes the rind has also the same origin and structure as in the stem. These details are shown in Fig. 6.

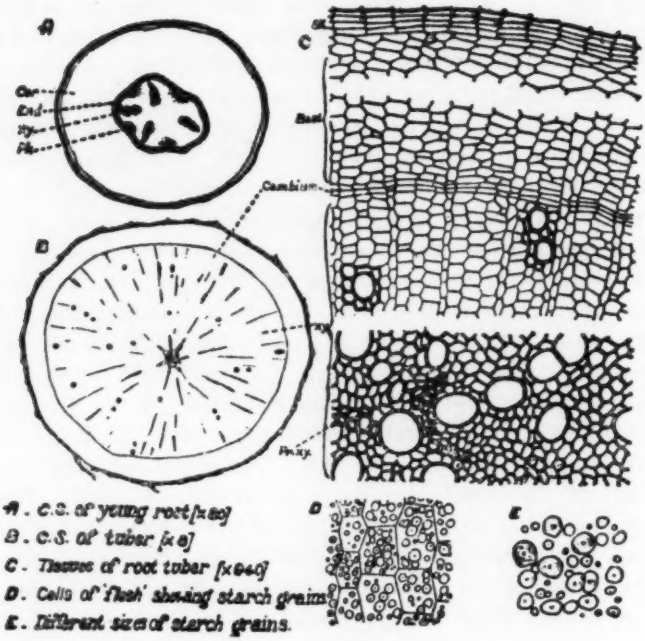
A
B
C
Cambium
A. C.S. of young root [x 25]
B. C.S. of tuber [x 6]
C. Tissues of root tuber [x 64]
D. Cells of 'flesh' showing starch grains
E. Different sizes of starch grains.

FIG. 6. Cross-Section of Tuber

III. VARIETIES UNDER CULTIVATION IN TRAVANCORE

Two principal forms of tapioca, the ' bitter ' variety (*Manihot utilissima*) and the ' sweet ' variety (*M. alpi*, Plon.) were recognised in earlier times in America. But specific differences between them being so slight and inconspicuous and the forms which have developed by centuries of cultivation so numerous and intergrading that this grouping is no longer followed. In Travancore '*aviyan*' and '*maravan*' both belonging to *M. utilissima* have been taken as two chief groups, the tubers of the former boiling easily while the latter required more time for boiling to remove the deleterious juice. The following are some of the chief varieties, the vernacular names signifying broadly the characters either of the stem or of the tubers.

Vella maracheeni, Chenkompan, Pacha aviyan, Anai maravan, Malai elavan, Ariyan, Rottivella, and Kanchavu ariyan.

With a view to ascertaining the nature and extent of variation in the indigenous varieties, an extensive collection was undertaken by the Econo-

mic Botany Section of the Department of Research. Seventy-three so-called varieties were collected from different parts of Travancore. Two varieties were brought from Mysore by the author and one Java variety was obtained from Bangalore. Of these 76 varieties 20 did not survive. The remaining 56 varieties were studied with reference to their morphological characters according to the scheme given below.

Scheme for description of the Plants

Plot No.
Number of the plant
Date of planting
Growth Habit:
- (*a*) Erect or Spreading
- (*b*) Branched or not

Colour of
- (*a*) Old Stem
- (*b*) Young Stem
- (*c*) Leaf-bearing portion

Colour of
- (*a*) Petiole
- (*b*) Stipule
- (*c*) Leaf
- (*d*) Budleaf

Date of Flowering
Colour of
- (*a*) Male Flower
- (*b*) Female Flower
- (*c*) Fruit
- (*d*) Fruit wings

Tuber—Colour of:
- (*a*) Skin
- (*b*) Rind
- (*c*) Flesh

Date of Harvesting
Number of Tubers per plant
Total weight of tubers
Percentage of starch
Percentage of HCN.

Method of Classification

Greenstreet and Lambourne (1933) have classified tapioca into two groups with reference to the colour of the cortex being either light green or dark green. As growth habit is a more permanent varietal character in tapioca a revised scheme of classification has been made by the author as given below. Careful observation of their external features has enabled an accurate estimate of the extent of variability of the characters possessed by these plants. Three more or less permanent varietal characters were taken as the basis for a classification of the recorded varieties. These are (1) colour of stem, petiole and tuber, (2) growth habit and (3) flowering or sterile.

The following scheme of classification has accordingly been evolved, reference to the registered varieties pertaining to each group being shown against each.

Classification of Indigenous Varieties of Tapioca:

GROUP I.—*Erect, Tall-Branching*—

Type 1.—Mature stem grey, petiole red, tuber skin white.
A—Budleaf green—*Nos.* 10, 12, 46.
B—Budleaf pink—*No.* 8.
C—Budleaf pink, male sterile—*Nos.* 4, 5, 36, 54, 73.

Type 2.—Mature stem grey with black tinge, young stem with red streaks. Petiole red, budleaf green, tuber skin brown, rind, red—*Nos.* 52, 63.

Type 3.—Mature stem brown, tuber brown.
A—Petiole red, budleaf pink, tuber rind reddish—*Nos.* 18, 47.
B—Petiole red, budleaf pink, tuber rind white—*Nos.* 7, 38.
C—Petiole red, budleaf green, tuber rind cream—*Nos.* 6, 28.
D—Petiole deep red, budleaf pink, tuber skin reddish brown, rind white—*No.* 9.
E—Petiole yellow along lower and red along upper side, budleaf green, tuber rind cream—*No.* 24.

Type 4.—Mature stem deep brown. Branches stunted and bushy. Petiole short yellow with reddish base. Tuber brown, long and with elongated stalk. Budleaf green. Male sterile—*Nos.* 30, 75.

GROUP II.—*Erect Low-Branching*—

Type 1.—Mature stem grey, tuber skin white, nodal swellings prominent close set.

A—Petiole red, budleaf pink—*Nos.* 3, 23, 39, 43, 67.
B—Petiole red, budleaf green—*Nos.* 1, 15, 40, 41, 48, 70, 76.
C—Petiole green, with reddish middle and pink base, budleaf green, male flowers sterile—*No.* 32.

Type 2.—Mature stem grey with black tinge, young stem green, petiole all green, budleaf green, tuber white—*Nos.* 21, 26.

Type 3.—Mature stem grey, young stem yellowish green, petiole yellowish green.
A—Budleaf green—*Nos.* 13, 35.
B—Budleaf pink—*No.* 45.

Type 4.—Mature stem brown, petiole red, budleaf pink, tuber brown—*Nos.* 11, 42, 49, 74.

Type 5.—Mature stem greyish black, petiole red, tuber skin white.
A—Budleaf pink—*Nos.* 17, 27, 65.
B—Budleaf green—*Nos.* 56, 66, 68.

GROUP III.—*Spreading, Profusely Branching.*

Type 1.—Mature stem reddish brown, petiole yellow, tuber brown,
A—Budleaf pink—*No.* 61.
B—Budleaf green, Male sterile—*No.* 62.

Type 2. Mature stem blackish brown, petiole yellow, budleaf green, tuber brown, rind and flesh yellow—*Nos.* 29, 51.

GROUP IV. *Tall, erect, rarely branching, Non-flowering. Stem greyish black, wood yellowish, tuber brown.*

Type 1.—Petiole black-red, budleaf green—*Nos.* 22, 37.

Type 2.—Petiole red on the upper and yellow on lower side with base red. Leaf-base prominent—*No.* 57.

IV. CULTIVATION OF TAPIOCA

Travancore is an agricultural country, nearly half of the population depending almost exclusively upon land for their livelihood. Of its total area of 7,662 sq. miles, the wet land along the west coast and the hills and forest along the east, take up nearly half this area so that only about 3,500 sq. miles of land are available for cultivation in Travancore. As this area is fragmented into small holdings, large-scale cultivation of extensive areas as in other countries is not possible here nor can a uniform method of cultivation be followed for any crop. Roughly about a fourth of this area or about 5,00,000 acres of land is now used for the cultivation of tapioca.

Tapioca plant can withstand long-continued and extreme drought but the seed canes need abundant moisture to sprout. The cultivation of this crop in Travancore is thus regulated with reference to the two monsoon seasons. In central and north Travancore the crop known as '*karkadaka kappa*' is harvested in the month of Karkadagam (July-August) and the land is prepared for fresh planting by October when the N. E. monsoon rains render suitable conditions for seed canes to sprout well. In south Travancore, however, cultivation commences in April or May depending on the S. W. monsoon for its water supply and the crop is harvested in January or February. As December to April are the dry months of the year, the central Travancore crop has to pass through the entire dry season. Although this is a serious disadvantage, the fact that this crop gets the full benefit of the entire S. W. monsoon rains during the period of maturity may perhaps be an advantage in tuber development. This however has to be ascertained by comparative yield trials.

The ground is prepared for planting by ploughing followed by a harrowing to smooth off rough edges of the field. The seed canes are cut in pieces 6–8 inches in length, and the cuttings are planted erect, usually two in each pit. The pits are usually 2½–3 feet apart. Cattle-dung and ash are the only manures, employed either in pits before planting or as basic dressing before ploughing the field. In some places planting is on small mounds 3 feet apart with three cuttings in each mound. Except for a weeding operation when the ground is carefully loosened after about a month, little or no care is given to the crop. Two or three buds grow from each seed-cane. These grow up and may become branched, developing leaves rapidly on the elongating axis. As the plant becomes mature all but a few stunted leaves at the top are shed. Harvesting is done at this stage either by digging up the tubers if the soil is hard or pulling up the plant with the tubers. The yield of tapioca is as variable as that of other cultivated crops, depending on the nature and fertility of the soil as well as the quality of the seed canes. On an average 2–2½ tons per acre is considered a fairly good return by the ryot.

V. Genetical Work on Tapioca

1. *Hybridisation—Intervarietal*

Tapioca is essentially a vegetatively propagated plant, although most of its varieties flower profusely and set seeds. In order to undertake genetical studies on this plant, the conditions necessary for the germination of its seeds had first to be ascertained. Seeds of three local varieties were collected and extensive germination trials were made in the Botany

Department. In the first set of experiments no seed germinated even after three months. The seeds were then subjected to certain pre-treatments. In one set of experiments the seeds were soaked for varying periods before sowing in pots. This too did not yield favourable results. In another set the testa at the hilum region of the seed was rasped before planting in seed-pans. Out of thirty seeds thus treated, one seed germinated. Soaking the seeds for 2–3 days maintaining a constant temperature of 35–37° C. was found more successful. Petridishes with moist sand and 4–6 seeds in each were left for one week in an incubator at a constant temperature of 37° C. The seeds responded to this treatment well, and within a week almost all seeds sprouted. At this stage the seeds were transferred to the field. On an average about 80% of seeds thus transplanted grew up into vigorous plants.

Having thus evolved a technique for hastening germination, hybridisation was taken up on eight varieties of tapioca grown in a small plot of land behind the University Office. Tapioca flowers being unisexual, cross-pollination is easy. Female flowers to be crossed were covered over by muslin bags early in the morning of the day they open. Usually these flowers open by about noon. Mature male flowers (of plants selected as male parents) are then collected in a dish with a little water just before these flowers open. Pollination of the 'bagged' flowers is then effected by carefully opening the muslin bag and rubbing the anthers of a male flower on the stigma of the female flowers. The bag is again tied round the female flower and the ovary is left undisturbed to ripen into fruit. Usually the fruits ripen from 80–100 days after pollination. The hybrid seeds obtained by reciprocal crosses of five varieties of tapioca were grown and the plants were studied with reference to their cytology and genetics by one of my research students, Mr. T. J. Koshy. Meanwhile Mr. A. Abraham, Economic Botanist, also undertook hybridisation work at Kayamkulam. Over 1,300 hybrid seeds belonging to 122 crosses were obtained by him. With the organisation of a Research Farm for tapioca at Trivandrum further work on this crop plant became possible. Over 700 plants from these hybrid seeds were planted for yield trials in April 1945. The weight of the tubers of each hybrid plant was recorded during harvest and a selection of 173 plants was made for further work. Of these, 91 plants with ten replications of each were planted in a plot 220 ft. × 32 ft., 41 plants with five replications of each in a plot 120 ft. × 14 ft., and another 41 plants each with five replications, in a third plot measuring 120 ft. by 14 ft. Plantings in these three plots have been in Balanced Incomplete Block experiments designed by the Statistical Department of

the University. On the basis of yield data 23 varieties have now been selected for the third year's cultivation, the average yield of each selected variety being over 10 lbs.* per plant. The outstanding morphological characters of these plants together with their tuber-weights in the first and second year's cultivation are shown in Table I.

TABLE I

Number of Hybrid	Parents	Average tuber wt. per plant		Morphological characters
		I year	II year	
		lbs. oz	lbs. oz.	*
114	28 × 27	5 5	9 9	B ls F
117	28 × 29	7 12	10 0	T E (F)
94	29 × 35	2 14	10 9	B ls F
96	do.	3 11	13 7	B ls (F)
99	do.	6 0	10 7	B ls F
177	do.	3 12	10 7	B ls F
195	do.	7 4	12 6	B ls F
228	29 × 45	4 2	12 1	B is F
239	do.	2 6	12 2	B ls F
105	29 × 47	3 11	19 5	B ls F
106	do.	7 7	10 8	T E (F)
107	do.	6 12	10 0	T E (F)
108	do.	6 10	12 8	B ls F
669	34 × 29	4 4	10 1	B ls F
666	do.	6 14	11 3	B ls F
808	do.	4 1	10 0	T E F
874	38 × 29	9 2	10 5	B hs F
895	do.	11 0	12 6	B hs F
896	do,	7 0	13 5	B hs F
408	do.	4 4	12 6	B ls F
894	do.	4 9	10 0	T E F
358	38 × 45	4 0	10 8	T E (F)
479	38 × 63	3 4	10 0	B hs F

* B–Branching ; E–Erect ; T–Tall ; ls–low-spreading ; hs–high-spreading ; F–Flowering ; (F)—Non-flowering.

By continued selection work on these lines it would be possible to make a final selection of a few which are markedly superior to the remaining in yield. These selected varieties of hybrids will be multiplied and made available to the ryot for extensive cultivation.

2. *Interspecific Hybridisation*

Tapioca has a closely related plant *Manihot glaziovii* (ceara-rubber plant) in Travancore. It is also a native of tropical America having been introduced in this country about the same time as tapioca. Before Hevea

* As some 4,500 plants are grown per acre, an average yield of 10 lbs. per plant corresponds to a yield of 20 tons per acre.

rubber plants became more popular for the rubber plantations of Travancore, ceara-rubber was under cultivation here, though on a limited scale. It is a medium-sized, spreading, quick-growing tree 40–50 feet high. Interspecific hybridisation between tapioca and ceara was undertaken by Mr. A. Abraham at Kayamkulam with a view to introducing, if possible, favourable genes into tapioca. Cross-pollination with ceara-rubber plant as female parent was not successful while from 391 crosses with 13 varieties of tapioca as female parent, 8 seeds were obtained. Three of them germinated and grew up as tall, robust plants. Of these, hybrid 34 × R* exhibited the phenomenon of gigantism more markedly than the other two, 63 × R and 29 × R. All of them were different from either the tapioca or the rubber parent. Hybrid 34 R was pulled out when 14 months old. Tubers of medium thickness, but longer and more numerous were seen developed on it. The other two plants are still growing in the Farm with a ceara-rubber plant nearby. These are shown in the adjoining photograph (Fig. 7).

The outstanding morphological characters of hybrid 63 × R are shown in Table II along with the characters of its parents for comparison.

TABLE II

Morphology	Parent No. 63	Hybrid 63×R	*M. glaziovii*
Growth habit ..	Erect, tall branching	Erect, very tall branching at 10'	A tall tree
Stem ..	Grey with nodal swellings	Reddish brown. No nodal swellings	Reddish brown. No nodal swellings
Petiole ..	Purple	Purple	Green
Leaf ..	7-lobed middle broad	3–7 lobed middle broad	5–7 lobed distal broad
Age at flowering ..	4–5 months	12–14 months	12–14 months
Male flower ..	Pale green	Pink	Green
Fruit ..	Dark green	Green with violet tinge at base. Pedicel violet	Deep green
Fruit wings ..	Prominent	Not prominent violet tinged	No wings
Tuber skin ..	Brown	Yellowish brown	Dark brown
,, rind ..	Pink	Pale pink	Cream

As there is marked variation in the shape of the leaves, a mature leaf from each of these plants is shown in Fig. 8 A.

During last year this hybridisation work was continued and 38 plants were obtained. They have also grown up into as robust plants as the first three plants. The female parents of these hybrids with the number of plants obtained in each cross are shown below:

* Ceara-rubber plant as male parent.

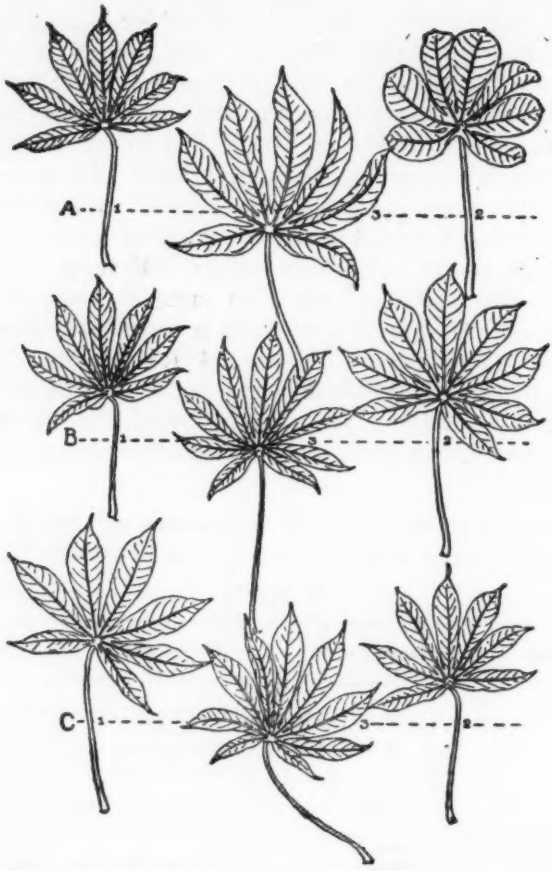

FIG. 8. Variation in leaves of A (1) Tapioca No. 63, (2) Rubber plant and (3) Interspecific Hybrid. B. (1) No. 27 tapioca (2) 34 × R Hybrid (3) 24 R × 27. C. (1) Tetraploid 3/10 (2) Diploid No. 63 (3) Triploid type.

29 × R	.. ..	9 plants	
32 × R	.. ..	2 „	(Male sterile)
36 × R	.. ..	5 „	(Male sterile)
38 × R	.. ..	5 „	
48 × R	.. ..	4 „	
51 × R	.. ..	1 „	
61 × R	.. ..	4 „	
73 × R	.. ..	2 „	
74 × R	.. ..	6 „	

Although all these plants have also flowered, plants derived from female parents Nos. 32 and 36 have only sterile male flowers. In their external appearance these hybrid plants are markedly different from either the tapioca plants or the ceara-rubber plant. The nodal protuberances characteristic of tapioca stems are absent in them although leaf-scars are well marked out. The colour of the stem is usually seen to approximate more closely the female tapioca parent than the stem of the rubber plant. The first few leaves of the plant resemble the rubber plant more closely than those of tapioca. It is significant however, that as these plants grow up, the leaves gradually become so transformed as to resemble more closely the tapioca type. Fig. 9 illustrates this aspect of hybrid growth very clearly. It is a photographic reproduction of three plants, a seedling of ceara-rubber (A), a hybrid seedling 74 × R, (B) and a young hybrid plant grown from a cutting of 34 × R, (C). The leaves of the ceara-rubber plant can easily be recognised by the 5-lobed obovate, close-set leaves. In the hybrid plant B, the lobes of the first formed leaves are obovate, but have acute tip. After forming about a dozen such leaves, they become narrower as seen in the upper leaves of hybrid B, approximating the leaves of tapioca. In the mature hybrid plant the leaves are closely similar to the tapioca type as seen in plant C, which is derived from a cutting of the hybrid.

Further work on these has been directed on (1) back-crossing, and (2) self-pollination.

3. *Back-crossing*

Using 29 R as male parent on variety No. 29 four seeds were obtained. Hybrid 34 R was back-crossed with two varieties similar to 34 (Nos. 27 and 63). The seeds obtained by this have been planted and two plants—one belonging to each parent—have grown up. Fig. 8 B shows a typical leaf of No. 27 (1) of hybrid 34 R (2) and of hybrid 34 R × 27 (3).

Outstanding morphological characters of these are shown in Table III.

TABLE III

Morphology	Hybrid 34 R (Male)	No. 27 (Female)	Hybrid 34 R × 27
Growth habit ..	Tall spreading in umbrella form	Low branching	Erect, not branching
Old stem ..	Light brown	Grey	Light grey
Young stem ..	Yellowish green	Deep green	Deep green
Petiole ..	Bright red	Red	Green with violet tinge
Stipule ..	Bifid, small	Bifid with violet base	Trifid, prominent
Budleaf ..	Pale green	Pink	Light green
Age at flowering ..	12–14 months	4–5 months	Not flowered

Hybrid 63 R did not yield any results in back-crossing. Extensive hybridisation work has also been conducted using these hybrid plants as male parents on a number of tapioca varieties as shown below:

Female Parent	No. of seeds
29 × 29 R ..	.. 9
29 × 34 R ..	.. 5
51 × 34 R ..	.. 6
61 × 34 R ..	.. 30
62 × 29 R ..	.. 1
62 × 34 R ..	.. 8
74 × 34 R ..	.. 1
78 × 34 R ..	.. 4
98 × 29 R ..	.. 7
98 × 34 R ..	.. 9
98 × 34 R ..	.. 9

The behaviour of these seeds will be studied during the ensuing season. One of the significant results of back-crossing has been the evolution of a few hybrid plants which are either non-flowering or self-sterile. The agricultural importance of evolving sterile varieties by this method in a vegetatively propagated crop plant like tapioca, needs further investigation. Although over 100 self-pollination trials were made, only one seed and that from 29 R has been secured. In another set of trials these hybrids were used as female parents. Tapioca variety No. 38 on 32 R produced 6 seeds, No. 29 on 36 R—2 seeds and No. 38 on 36 R—2 seeds. Detailed genetical study of these different categories of hybrid seeds will be undertaken during the ensuing cultivation.

Interspecific hybridisation work on the lines detailed above appear to be very fruitful. However promising the present results, the final selection of suitable strains has to await further yield trials. The tubers formed in them are edible though more fibrous or woody. Owing to the vigour in growth and the prolific tuber production in them, one can reasonably hope for promising results in this line of work.

4. *Evolution of Polyploid forms*

The diploid number of chromosomes in *M. utilissima* is reported to be 36. Recent genetical work has established the fact that by inducing duplication of chromosome sets in certain cultivated plants, substantial increase in yield can be obtained. Inducing duplication of chromosomes thus appeared another fruitful line of work for tapioca. By repeated trials at Kayamkulam it was found that .5% solution of colchicine boiled

with agar-agar was effective on young buds. The solution was applied nine times at intervals of three hours on buds of five varieties of tapioca. On variety No. 10 the solution was applied on ten of its cuttings. Four of these developed forms markedly different from one another and also the mother plant. They are recorded under numbers 2/10, 3/10, 8/10 and 10/10. Of these 8/10 is non-flowering while 3/10 and 10/10 are profusely flowering and 2/10 flowering only sparsely. The chromosome number of 2/10 has been ascertained as 72 (4 *n*) by Mr. A. Abraham. Its tubers are stouter but the rind is at least twice as thick and the starch grains much larger than in normal types, indicating that the chromosomes have been duplicated. Apparently divergent forms were obtained by this method on variety No. 3. Two distinct forms obtained from it were

FIG. 11. Variation in leaf forms of Polyploids

TABLE IV

Morphology of Polyploids

Polyploids and normal derivatives	Growth habit	Old stem	Petiole	Budleaf	Shape of leaf	Colour of leaf	Flowering or not	Tuber characters	
								Skin	Rind
No. 10 normal	Erect low branching	Grey	Red	Pink	Elliptic	Green	Flowering	White	Cream
Polyploid 2/10	Erect low branching	do	Red distal, yellow towards the base	Green	Obovate	do.	do.	Brownish	Very thick reddish
Polyploid 3/10	Erect low branching	Light brown	Red	Deep pink (violet)	Elliptic	Deep green	do.	Brown	White
Polyploid 8/10	Erect tall branching	do.	do.	Chocolate	Elliptic	Green	Not flowering	White	White
No. 26 normal	Erect low branching not vigorous	Grey	Yellow	Green	Elliptic	Green	Flowering	White	White
No. 26 polyploid	Erect tall branching, vigorous	do.	do.	do.	Obovate	Thick and deep green	Not flowered	Brownish	Cream
No. 32 normal	Spreading	do.	Yellow with red at top and base	do.	Elliptic	Green	Flowering	White	Cream
No. 32 polyploid	Erect low branching robust	do	do.	Green	Obovate	Thick and deep green	do	White	White
No. 36 normal	Erect low branching	Grey with brown tinge	Red	Pink	Lanceolate	Green	do.	White	Cream
No. 36 polyploid	Erect low branching	do.	do.	do	Obovate	Thick and green	do.	do.	do.

found very promising in yield. These are included as $3P_1$ and $3P_2$ along with other high yielding varieties for further yield trials (*vide* Table II). Variety numbers 26, 32 and 36 were also subjected to this treatment and the forms derived from them are under observation. Fig. 10 is a photograph of a normal No. 32 plant (N) with a polyploid (P) derived from it. Variation in leaf-form in polyploid types in the four varieties detailed above is shown in Fig. 11.

Owing to the minute size of chromosomes and the poor reaction of cells to most of the known fixatives, the cytology of these polyploid forms has not yet been fully worked out. The marked variation of these forms in regard to external characters from their parent types is, however, indicative of chromosomal changes. The outstanding morphological characters of these forms in relation to their parent types shown below in Table IV would give an estimate of the extent of such variation. As in the case of hybrids, continued selection work based on yield data should enable ultimate selection of outstanding forms for propagation.

5. *Evolution of Triploids*

The possibilities of evolving improved strains of cultivated plants by increasing their chromosome numbers are not fully known; but the improvements achieved in triploid varieties of apples and pears (Nilsson-Ehle, 1938) justify the hope that evolution of triploid varieties in tapioca may lead to the improvement of this crop plant. True tetraploid (4x) forms when crossed with diploid (2x) varieties give rise, among other methods, to what are known as triploids (3x). Although it has not been established that the colchicine induced forms obtained from tapioca are definitely tetraploids, the effect of crossing these plants with the varieties now under cultivation (diploid types) was worth investigation. A start has therefore

TABLE V

Morphology	Diploid	Triploid? 3/10 × 63	Polyploid? 3/10
Growth habit ..	Tall branching	Non-branching	Tall branching
Old stem ..	Grey with violet tinge	Light brown, nodes close set	Deep brown, node swellings prominent
Young stem ..	Green with red streaks	Dark green with red streaks	Yellowish green
Petiole ..	Red with violet tinge	Bright red drooping	Deep pink
Stipule ..	Prominent, unbranched	3-fid with red base	Lobes with red base
Leaf ..	Broad middle	Broad middle	Broad distal
Budleaf ..	Green	Pale green with violet tinge	Pink
Age at flowering	4–5 months	Not flowered	6–8 months

been made with polyploid 3/10 as female parent and varieties 27, 28, 38 and 63 as male parents. 10 plants were obtained and they are under observation in the Farm. A typical leaf each of the tetraploid, the diploid and the triploid is shown in Fig. 8 C. The morphological features of these plants are given in Table V.

6. *Evolution of pure varieties of Tapioca*

The genetical behaviour of the different varieties of tapioca as also the large number of forms now cultivated in Travancore would make one

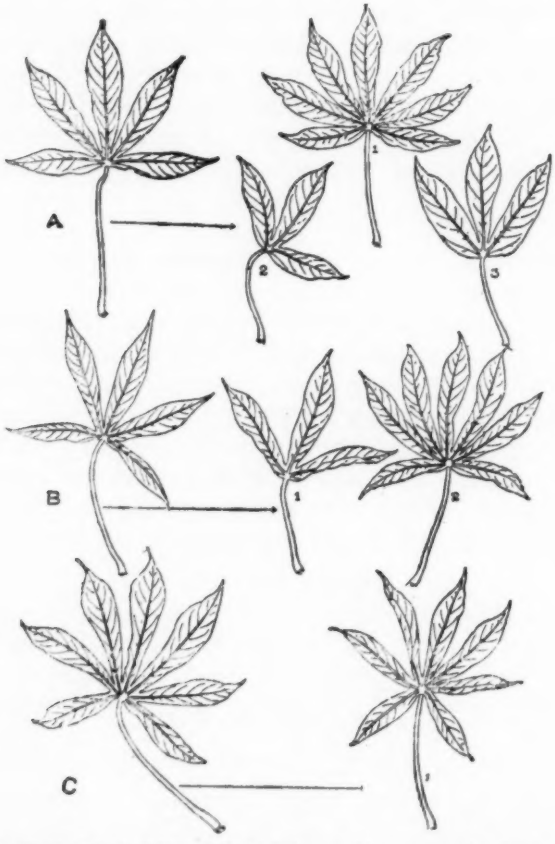

FIG. 12. A. Typical leaf of No. 47 1, 2, 3 types of leaves seen in the progeny of No. 47. B. Typical leaf of No. 49 1, 2 types of leaves seen in the progeny of No. 49. C. Typical leaf of No. 74 1-typical leaf of plant of the progeny varying in external characters from No. 74.

suspect if all of them are true varieties. It is likely that chance hybridisation occurring in nature would become perpetuated as the plant is vegetatively propagated. One of the fundamental problems for solution therefore is to find out how many of the indigenous types of tapioca are true varieties breeding true to parental types and how many of them are only hybrids. Continued self-pollination for successive generations of hybrid progeny would enable segregation of pure strains from hybrids. A start was therefore made this year on four varieties (Nos. 38, 47, 49 and 74) by selfing them in order to study their genetical behaviour. 19 plants were obtained from No. 38, 6 from No. 47, 3 from Nos. 49 and 2 from No. 74. Segregation was clearly seen in the progeny of all of them. Extent of variation of leaf form characteristic of the different groups into which the progeny of these can be classified is shown in Fig. 12. Outstanding morphological characters of the prominent types formed by this method are shown in Tables VI and VII.

TABLE VI

Morphology	Normal No. 47	Type 1	Type 2	Type 3
Growth habit ..	Tall branching	Tall branching	Profusely branching	Low branching
Old stem ..	Light brown	Brown	Dark brown	Brown with white streaks
Petiole ..	Red, middle yellowish	Bright red, middle yellowish	Light red, middle yellowish	Light red, middle yellowish
Leaf ..	5–7 lobed	3–7 lobed	3–lobed	7–lobed
Age at flowering	4–5 months	8–9 months	6–7 months	Not flowered
Male flower ..	Yellowish green	Sterile	Yellowish white	
Fruit wings ..	Prominent pink	Pink, wavy stalk pink	Pink, wavy stalk pink	

TABLE VII

Morphology	Normal No. 49	Type 1	Type 2
Growth habit ..	Tall branching	Erect, not branched	Profusely branching
Old stem ..	Grey	Brown	Brown
Young stem ..	Green	Yellowish green	Yellowish green
Petiole ..	Upper red, lower yellow	Red with middle yellow	Red with middle yellow
Stipule ..	3–fid. red base	3–fid, red base	2–fid, red base
Leaf ..	5–7 lobed	7–lobed	2–3 lobed
Budleaf ..	Pink	Green	Green
Age at flowering	4–5 months	Non-flowering	6–7 months
Male flowers	Greenish white with reddish yellow disc	..	Open only partially, greenish
Female flowers	Greenish yellow	..	Open partially by slit between perianth lobes
Fruits ..	Numerous	..	Few
Tuber ..	White, thick	Reddish	Reddish

'Selfing' was repeated on one of the six plants of No. 47 and 30 seeds have been obtained. One of the progeny of No. 49 by selfing has given 12 seeds. In addition, varieties 17, 27, 56 and 98 have also been selected for selfing and 4, 143, 41 and 146 seeds respectively have been collected. These seeds will now be planted for further study.

VI. Chemical Composition of the Tubers

According to an analysis made in the Bureau of Chemistry of the U.S. Department of Agriculture (Tracy, 1903), the chemical composition of dry tapioca tubers is as follows:

Constituents	per cent.
Moisture	5.76
Ether extract ..	.42
Crude fibre ..	5.08
Pentosan	2.63
Starch	64.28
Protein	2.98
Ash	1.96
Sugars, soluble cellulose, etc. ..	16.89

The proportion of water in the fresh tubers varies with the nature of the soil and the time of harvesting. On an average about 66 per cent. of the tuber is water. Hence the constituents of the fresh tubers will be:

Moisture	66.00
Ash	.71
Protein	1.07
Crude fibre ..	1.83
Starch	30.24
Ether extract ..	.15

As a food stuff the carbohydrates are largely in excess in tapioca with a nutritive ratio 1 : 28.5 instead of 1 : 7 which is accepted as the standard for a balanced diet. Fortunately Travancore does not lack fish and other nitrogenous food stuff rich in proteins. It should therefore be possible by a judicious combination of these with tapioca to have a balanced diet.

The results of analysis of tubers of 27 varieties of tapioca, conducted in the Division of Applied Chemistry (Public Analyst's Section) of the Central Research Institute of the Department of Research of the University, are shown below:

Sl. No.	No. sample	Composition of raw tubers %			Composition of edible portion (moisture free)			Starch content of raw tuber %
		Rind	Edible portion	Moisture	Starch	Ash	HCN	
1	1/10	14·4	85·6	68·7	81·7	2·10	·034	21·9
2	2/10	22·5	77·5	75·4	85·7	2·80	·173	16·4
3	3/10	13·0	87·0	67·4	86·2	1·65	·050	24·5
4	8/10	16·1	83·9	71·6	83·4	1·75	·064	16·6
5	10/10	14·1	85·9	68·7	81·7	2·15	·042	22·0
6	21	14·2	85·8	60·5	86·8	1·95	·031	29·4
7	22	18·3	81·7	60·1	82·9	1·80	·043	27·0
8	26	16·5	83·5	65·6	83·7	1·45	·039	24·0
9	28	16·0	84·0	74·8	81·1	2·10	·051	17·2
10	29	18·2	81·8	61·7	85·0	1·95	·018	26·6
11	30	21·1	78·9	53·8	82·9	2·60	·032	30·2
12	32	14·1	85·9	58·7	90·1	1·70	·023	32·0
13	35	11·5	88·5	66·8	85·9	2·35	·018	25·3
14	36	19·7	80·3	70·6	82·4	2·05	·042	19·4
15	38	16·1	73·9	58·6	81·7	1·85	·016	25·0
16	47	17·9	82·1	72·8	82·4	1·70	·088	18·4
17	49	18·6	81·4	67·5	84·8	1·75	·027	22·5
18	54	15·5	84·5	67·2	83·4	1·80	·046	23·1
19	56	18·9	81·1	62·8	85·6	1·80	·039	25·8
20	57	17·1	82·9	60·8	78·1	2·10	·059	25·4
21	61	15·2	84·8	67·6	85·8	1·75	·024	23·6
22	62	15·0	85·0	63·2	85·3	1·85	·022	26·7
23	63	17·8	82·2	58·5	85·1	1·35	·023	29·0
24	73	15·1	84·9	65·2	86·8	1·55	·028	25·6
25	74	15·7	84·3	68·5	—	—	—	—
26	75	18·1	81·9	55·2	84·4	1·80	·037	31·0
27	98	15·9	84·1	59·8	80·6	1·85	·045	27·2

Six out of the 27 samples have hydrocyanic content more than 50 mgm. per 100 gms. of dry tuber and it is significant that three of these are in polyploid forms. How far inducing polyploidy in this plant is associated with increase in HCN content is a matter for further investigation. It is also worth recording how one of the polyploid forms, 2/10 with a tuber rind about four times the normal thickness showed 173 mgm. of HCN. This should mean that there is a probable correlation between rind thickness and HCN content in these tubers.

VII. Summary

By the application of genetical methods detailed above, a good number of new strains of tapioca and tapioca × ceara hybrids have been produced. These strains are grown for yield trials with a view to selecting high yielding strains therefrom. The selected strains will soon be made available to the ryot for cultivation. Side by side with this line of investigation, experiments are also undertaken to ascertain (1) best mode of planting seed-canes, (2) optimum spacing for planting, (3) number of

plants per pit, (4) effective manures for the crop and (5) best period for harvesting. All these experiments are conducted on statistical design furnished by the Department of Statistics in the University. Owing to the fact that this line of work pertains to the agronomical aspects of this crop, it is proposed to embody its results in a separate paper. Meanwhile the above account of the applications of genetical methods for evolving better strains of tapioca, is presented as the first paper from the Tapioca Research Farm, in order to stimulate further work on this important crop plant calculated to improve its cultivation in Travancore.

In conclusion the author wishes to record his grateful appreciation of the interest evinced by Rajyasevapravina Dr. K. L. Moudgill, Director of Research, in promoting this research work on tapioca. He also wishes specially to acknowledge the valuable contribution made by Mr. A. Abraham, Economic Botanist now on deputation for advanced training in America, and the assistance rendered by the staff of the Research Farm, in the progress of this work.

Thanks are also due to Sachivottama Sir C. P. Ramawamy Aiyer, Dewan of Travancore and Vice-Chancellor of the University, for the munificient endowment of Rs. 1,000 a month from which this work is being financed.

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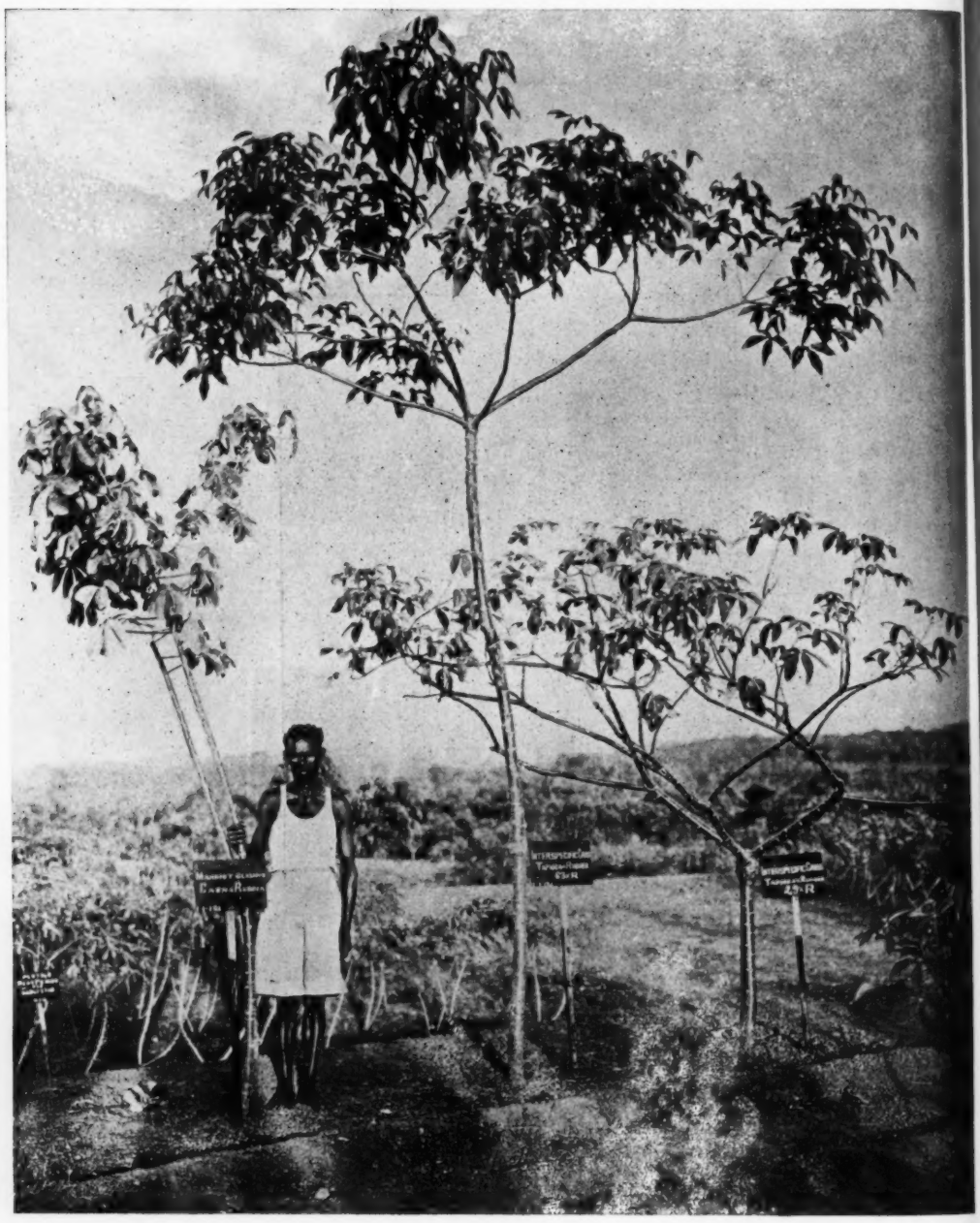
Fig. 4. Seedlings of Tapioca.



A B C

Fig. 7. A. The Ceara-rubber plant. B. Hybrid 63 × Rubber plant.
C. Hybrid 29 × Rubber plant — Age 18 months.

Fig. 9. Photograph of a Ceara-rubber plant (A) and two hybrid plants obtained by ceara × tapioca cross.

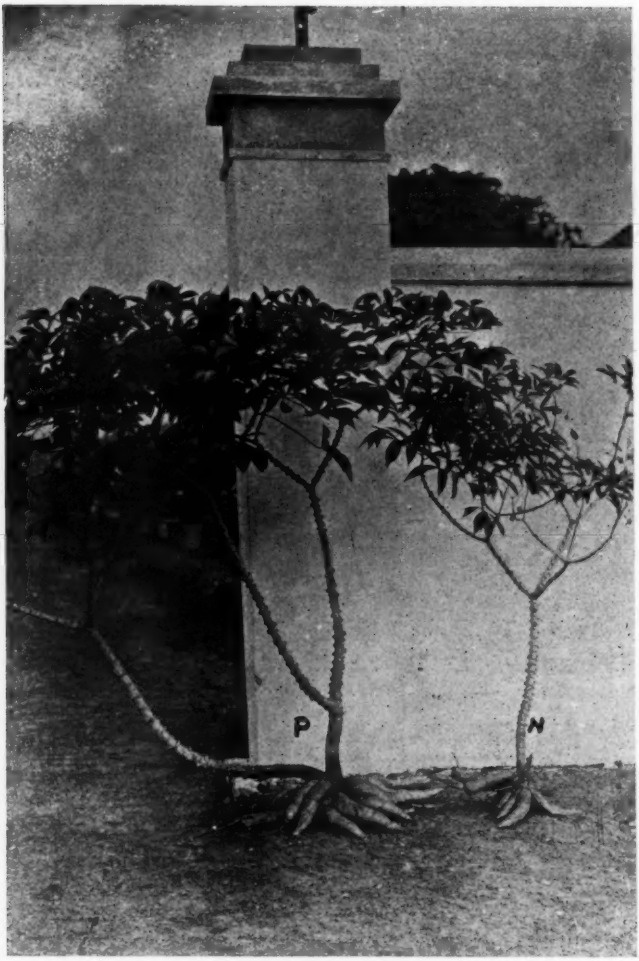


Fig. 10. Polyploid and normal tapioca plants.

Explanation of Text-Figures and Plates

Fig. 1. The tapioca plant × 1/20. The soil level is shown by the line at base.

Fig. 2. Young plant growing from a cutting × 1/8. Two buds have sprouted.

Fig. 3. Flowers and fruit of tapioca × 1. A. Inflorescence. B. 1. Female flower, 2. L. S. of same. 3. Ovary showing basal disc. 4. C. S. of ovary, 5. L. S. of ovary showing obturator passing through micropyle of the ovule. 6. Seed showing caruncle C. 1. Male flower. 2. Perianth removed showing the two whorls of flowers. 3. One stamen enlayed. 4. Disc.

Fig. 4. Photograph showing three seedlings (Nat. size).

Fig. 5. A. Macroscopic view of the cut end of stem. scl–sclerenchyma. B. Microscopic view in T. S. × 240 *Col.*—Collenchyma scl Sclerenchyma, *l. v.* latex vessel *xy.* xylem *p.* pith. C. Latex vessel seen in tangential view *l. v.* latex vessel.

Fig. 6. A. T. S. of young root. *Cor. Cortex, End.* Endodermis, *Xy.* Xylem, *Ph.* Phloem; B. Macroscopic view of the cut end of tuber. C. Microscopic view of T. S., *sk.* Skin. *xy.* Xylem; *Pr. xy.* Protoxylem. D. Starch grains in cells of tuber. E. Different sizes of starch grains.

Fig. 7. A. Ceara rubber plant. B. Interspecific hybrid 63 × Rubber Plant. C. Hybrid 29 × R. Age 18 months.

Fig. 8. Variation in leaf forms in Interspecific hybrids.

Fig. 9. Photograph of young ceara rubber and interspecific hybrids. A. Ceara rubber plant. B. Hybrid seedling 74 R. C. Cutting from hybrid 34 R.

Fig. 10. Photographs. N. Normal tapioca plant. P. Polyploid from derived from it.

Fig. 11. Variation in leaf forms of polyploids. Leaf forms of polyploids are shown on the right and normal plants on the left. Numbers represent variety numbers.

Fig. 12. Variation in leaf forms in the self-pollinated progeny of A. Leaf of No. 47 1, 2, 3 represent types of leaves found in the different plants obtained by selfing it. B. Leaf of No. 49. 1 and 2 represent types of leaves in the progeny and C. A leaf of No. 74, with type of leaf in one of its progeny.

A NEW RUST ON *DALBERGIA PANICULATA* ROXB.

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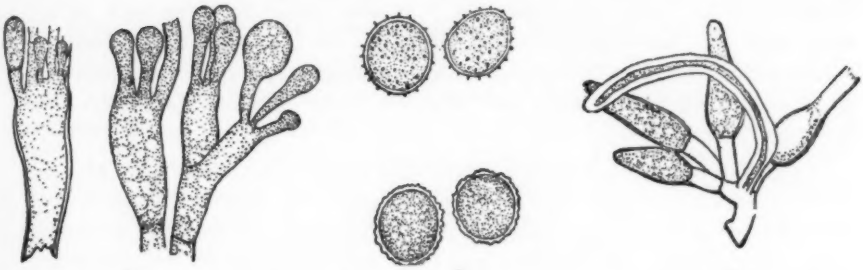
(*Mycology Section, Agricultural Research Institute, Coimbatore*)

Received June 20, 1947
(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

In 1946 the writers collected a rust on *Dalbergia paniculata* Roxb. from Walayar (Malabar District, South India). An examination of the fresh material of the rust indicated that it was different from those already recorded on related hosts and it is described below as being new.

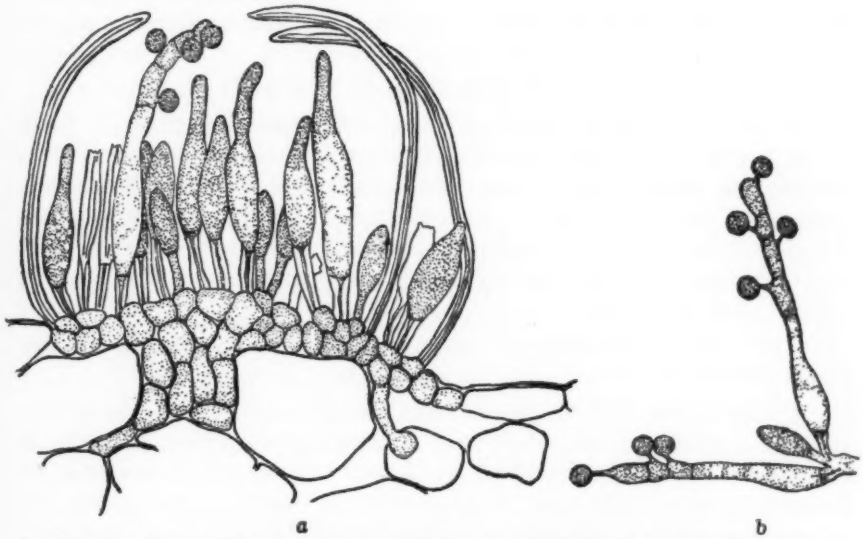
The uredia are hypophyllous or caulicolous, small and bright yellow. The sori develop just below the epidermis which is burst through by the sporogenous cells. The urediospores are globose or subglobose, stipitate, borne on short stalks which originate from the apices of cylindric branched or simple basal cells (Text-Fig. 1 *a*). Each basal cell gives rise to varying numbers of pedicellate urediospores. Two kinds of urediospores can be distinguished. One type has thin hyaline wall with prominent echinulations while the other has a thicker wall with less pronounced echinulations. Both have yellow contents. The proportion of the two kinds of spores varies in different sori and sometimes only the second type is present. The spores measure 15× 13 μ (12–19× 9–14 μ). On the twigs also the sorus develops below the epidermis and bursts through this layer exposing the spores. In the tissues of the branch the hyphæ are seen to extend as far as the pith region (being present in the cortex and vascular tissue also) which suggests that the mycelium may remain in the tissue producing new crops of sori for a long time.

Telia appear as buff to ochraceous buff, waxy, pulvinate, gregarious crusts amphigenously on the leaves and sometimes on the stem. The teliospores are seen in old uredia also. The exact place of origin of the telium is difficult to determine. It seems to be intra epidermal forming a two to three-layered tissue from which basal cells project out above the level of the epidermis. There is no evidence of the epidermis being pushed aside or burst through. It is difficult to clearly differentiate the epidermal cells in the region of the sorus, but vague outlines of the epidermal cells containing the lower cells of the sorus can be made out in portions of the sorus. Each basal cell gives rise to varying numbers of stipitate, oval to clavate, one-celled smooth and thin-walled teliospores. Incurved thick-walled paraphyses with narrow central lumen are found along the margin and



TEXT-FIG. 1. (*a*) Basal cells of uredia, (*b*) Uredispores × 500, (*c*) Paraphysis and Feliospores from common basal cell × 300.

other portions of the telia. The origin of the paraphysis can be traced to the same basal cell which produces the teliospores (Text-Fig. 1 *c*). The teliospores measure 15–30× 10–15 μ (average 19× 12). They germinate immediately producing promycelia which are direct outgrowths of the apices of the spores. The promycelium is four-celled and one round basidiospore is produced from each cell. The rust is a hemi form since it has only II and III stages.



TEXT-FIG. 2. (*a*) Section through a telium × 530 (*b*) Germinating teliospores × 350.

Maravalia achroa (Syd.) Arth. and Cumm. has been recorded on *Dalbergia sissoo*. Type specimen of this material was obtained through the courtesy of Mr. J. F. Dastur from the Indian Agricultural Research

Institute, New Delhi and examined. It was found that the telia of this rust were not paraphysate. The formation of the basal cells producing clusters of teliospores was not evident. Cylindrical basal cells developing groups of urediospores were not seen. In these characters it differs from the rust under study.

The formation of intra-epidermal telia suggests an affinity to *Mainsia*. Though Jackson (1931) has recorded this genus on species of *Rubus* only, Thirumalachar (1947) has described *M. pterocarpi* on *Pterocarpus marsupium* from South India. In *Mainsia* however, the urediospores develop singly, and not in clusters from free basal cells. Further the epidermal cells in the vicinity of the sori are said to be considerably hypertrophied in *Mainsia*. The rust on *D. paniculata* differs from *Mainsia* in these respects. The production of urediospores and teliospores in clusters from free basal cells indicates relationship to *Scopella*. But in the latter genus the sori are subepidermal and paraphyses have not been recorded in any of the species, whereas in the rust now described the telia are intra epidermal, and paraphysate. Thus it does not conform to any of the known genera of rusts and is therefore accommodated in a new genus *Scopellopsis* because of its resemblance to *Scopella* in the development of free basal cells bearing groups of spores in the uredia and telia. The rust on *D. paniculata* is described as *Scopellopsis dalbergiæ*.

Scopellopsis gen. nov. Ramakrishnan, T. S. and K.

Pycnia and *æcia* not known; *uredia* subepidermal, erumpent, hypophyllous and caulicolous; *urediospores* subglobose, echinulate, pedicellate, produced in clusters from stout almost cylindric basal cells; *telia* amphigenous or caulicolous, waxy, intra-epidermal in origin, projecting above the epidermis; *teliospores* stipitate formed in clusters from free basal cells, oval to clavate, one-celled, germinating *in situ*; paraphysate, with incurved almost solid paraphyses.

Type Species: *Scopellopsis dalbergiæ* Ramak., T. S. and K. on *Dalbergia paniculata*.

Pycnia et *æcia* ignota; *uredia* subepidermia, erumpentia, hypophylla, caulicola; *urediosporidia* subglobosa, echinulata, pedicellata, producta in racemis ex cella crassa cylindrica simplici vel ramosa; *telia* amphigenia, vel caulicola, ceracea, plurimum intraepidermia, projicientia super epidermem; *teliosporidia* stipitata, formata in racemis singulis cellis, ovalia vel clavata, unicellata, germinantia *in situ*; paraphysata, paraphysibus incurvatis, ferme solidis.

Species typica *Scopellopsis dalbergiæ* Ramak., T. S. and K., In vivis foliis et ramis *Dalbergiæ paniculatæ*.

Scopellopsis dalbergiæ Ramakrishnan, T. S. and K., sp. nov.

Pycnia and *æcia* not known; *uredia* bright yellow, hypophyllous sometimes caulicolous, subepidermal, erumpent, minute, gregarious, pulvinate urediospores globose to subglobose, echinulate, with hyaline wall and yellowish contents, 15× 13 μ, stipitate, formed in clusters from simple or branched, stout, cylindric cells; telia amphigenous, caulicolous, ochraceous buff, waxy, intraepidermal, spores projecting far above the epidermis; *teliospores* stipitate, formed in groups from free basal cells, oval to clavate 19× 12 μ (14–30× 9–15 μ) germinating immediately *in situ*; paraphysate, with almost solid incurved paraphyses.

On living leaves and stem of *Dalbergia paniculata* Roxb. Walayar (Malabar), 31–12–46 T. S. Ramakrishnan and K. Ramakrishnan.

Pycnia et *æcia* ignota; *uredia* lucida flava, hypophylla et caulicola, subepidermia, erumpentia, minuta, gregaria, pulvinata; *urediosporidia* globosa vel subglobosa, echinulata, murus hyalini, contenta flavida, 15×13 μ, stipitata, producta in racemis ex singulis cellis, crassa, cylindrica, simplici vel ramosa; *telia* amphigena, vel caulicola, silacei lutei colores, ceracea, intraepidermia, sporidia projicientia super epidermem; *teliosporidia* stipitata formata in racemis singulis cellis, ovalia vel clavata, 19× 12 μ (14–30×9–15 μ) germinantia *in situ*; paraphysata, paraphyses incurvatis, ferme solidis.

In vivis foliis et ramis *Dalbergia paniculata* Roxb. Walayar (Malabar) 31–12–1946. T. S. Ramakrishnan et K. Ramakrishnan.

Type specimens of the rust have been deposited in the Herbarium of the Government Mycologist, Coimbatore, and in Herb. Crypt. Ind. Orient., New Delhi.

Acknowledgment

The writers are indebted to Dr. B. B. Mundkur of New Delhi and Dr. G. R. Bisby of the Imperial Mycological Institute, Kew, for their valuable criticisms and suggestions. They are also thankful to Mr. J. F. Dastur for kindly supplying the type specimen of *Maravalia achroa* (Syd.) Arth. and Cumm. Rev. Fr. Singarayar, Coimbatore, was kind enough to translate the diagnosis into Latin.

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REVISION OF A RUST ON *OLDENLANDIA* SPP.

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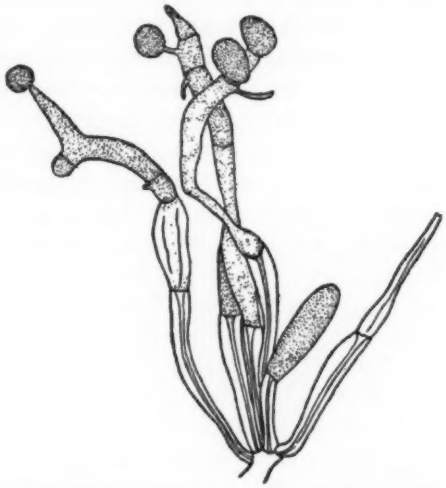
(Communicated by Dr. T. S. Sadasivan, M.Sc., Ph.D., F.A.Sc.)

In 1946 the writers collected rusts on *Oldenlandia stylosa* O. Kze. (*Hedyotis stylosa*, Br.) and *Oldenlandia articularis* Gamble (*Hedyotis articularis* Br.). The rusts on these two hosts agreed with the description of *Chrysocelis ascotela* (Syd.) Thirumalachar. An examination of the fresh material of these specimens indicated that the taxonomy of this rust was in need of revision.

Oldenlandia stylosa O. Kze. is affected by a rust on the Nilgiris and Pulneys. One or more orange yellow spots which on drying turn black on the upper surface are formed on the leaf. On the upper surface of the spot several reddish brown to red pycnia are present. These are sometimes hypophyllous though more often they are epiphyllous. The pycnia are sub-globose, subepidermal, deep-seated, and paraphysate with the paraphyses projecting out of the ostiole (Plate IX, Fig. B). On the lower surface of the spots numerous crowded telia are seen. When fresh a wax-yellow to golden yellow colour is presented after the germination of the teliospores. Each telium is sunk in the tissue of the leaf. A peridium is lacking. The teliospores develop in oval broad cavities of the mesophyll (Plate IX, Fig. C). They are one-celled clavate to cylindrical $54 \times 13 \cdot 6\,\mu$ ($37–70 \times 9–18\,\mu$), pedicellate, thin-walled and filled with yellowish contents. The spores originate from a mass of hymenial cells, in the topmost layer of which the cells are laterally free and from each of which two, or more teliospores are developed. The spores of a cluster are of varying ages and all stages from initial formation to spores that have collapsed after germination can be seen in each group originating from a basal cell. Each spore has a pedicel which lengthens as the spore matures and may attain a length of $40\,\mu$ or more. When scrapings of the spores are examined under the microscope it is often seen that the pedicels are not complete but get partially broken. The pedicel is hyaline and has a central protoplasmic strand with clear gelatinising portion all round. Such pedicels have been described by Cummins (1940) for *Scopella bauhinicola*. Thus the spores are pedicellate and are produced in groups from laterally free basal cells.

The teliospores germinate *in situ* as soon as they are fully developed and at this time the stalk elongates to its maximum so that the promycelium is pushed out of the leaf tissue. The promycelium is the direct prolongation of the apex of the spore and does not come out through a germ pore. It is yellowish in colour, 4-celled and from each cell a big oval or round, thin-walled, light yellow basidiospore is produced on a sterigma. The protruding promycelia get reflexed on the epidermis and masses of these give a waxy appearance to the telia. The teliospores do not come out of the leaf at all but only the promycelia project out in a mass through a wide opening. The spore collapses after germination.



TEXT-FIG. 1. A cluster of teliorpores of *Scopella ascotela* froma basal cell × 300.

This rust was first described by Sydow (1935) as *Blastospora ascotela*. Mains (1938) examined the specimen again and renamed it *Maravalia ascotela*. Thirumalachar (1942) studied it in greater detail and transferred it to the genus *Chrysocelis* as *C. ascotela*. Now our examination of fresh specimens collected at Ootacamund in October 1946 has shown the necessity for a further revision of the genus of the rust.

This rust is not *Blastospora* as is evident from the formation of the telia subepidermally. Thirumalachar includes the rust under *Chrysocelis* which has sessile teliospores, for he does not concede that the cell below the spore is a pedicel though he states that this elongates and reaches 40–70 μ in length and " simulates a pedicel ". In our opinion the cell below the spore is definitely a pedicel, reaching its maximum length when the spore germi-

nates. That it is a pedicel is shown by its structure. The central protoplasmic strand with hyaline gelatinising outer portion in this cell is indicative of its being a pedicel and not any other structure. Such pedicels have been noticed in *Scopella* by Cummins (1940). Further it is hyaline and the spore is coloured. With the collapse of the spore after germination the lower cell does not make any further growth but disintegrates in the same manner as remnants of pedicels often do. Thirumalachar has stated that the spore is hyaline and he has not observed the difference in colour between the spore and its pedicel. The colour of the spore is conspicuous in fresh specimens; but in old herbarium specimens, or two or three months after collection the colour is lost and this may be the reason why the colour has not been described by earlier authors. Mains (1938) has also described the spore as hyaline. Since the teliospores are prominently pedicellate the rust cannot be *Chrysocelis*. In *Maravalia* the teliospores are produced singly from the cells of a compact hymenium (Mains, 1939). In the rust under study examination of microtome sections and dissected telia showed that the teliospores are formed in groups of varying numbers, each group developing from a basal cell which is laterally free (Plate IX, Fig. A). That the cells are laterally free can be clearly seen in the photomicrograph of a cluster where a small spore is developing from a side of the basal cell which will be possible only if the basal cells are laterally free. Each cluster contains spores in different stages of development. For these reasons this rust cannot be included in the genus *Maravalia*. Judging from the characters of the telia and the teliospores it must be transferred to the genus *Scopella*. Mains (1939) who founded the genus has stated that in *Scopella* the pycnia are subcuticular and hemispherical while in this rust they are subepidermal and subglobose. This difference need not be a serious objection to include this rust under *Scopella*. Instances are known in other genera [*e.g.*, *Ravenelia* (Arthur, 1934)] where both kinds of pycnia have been observed in the same genus.

The distinction between *Scopella* and *Maravalia* rests mainly on, (1) the compactness or lateral freedom of the basal cells and (2) the formation of one or more teliospores from each basal cell. Cummins (1940) states that the basal cells are subject to variation and that there is no rule by which one can definitely decide when basal cells cease to be basal cells and become part of a compact hymenium. Considering the variation that may be expected in the hymenial layers and taking into account that we are dealing with living organisms in which machine-made uniformity cannot be expected, it is quite possible that *Maravalia* and *Scopella* are merged into one and the same genus, at a later time. For the present this merger is not attempted

and as the rust does not fit in with Mains' emended diagnosis of *Maravalia* but agrees more with *Scopella* it is thought fit to place this rust in the latter genus and revise the name as *Scopella ascotela.*

A similar rust was found infecting the leaves of *Oldenlandia articularis* in the neighbourhood of Ootacamund and the Agricultural Research Station, Nanjanad, Nilgiris. Only telia were present and these formed deep-pink, waxy, raised patches on the lower surface of the leaves. Corresponding brown areas became visible on the upper surface at a later stage. Telia are subepidermal and sunk in the tissue. Teliospores are one-celled, deep orange coloured with thin hyaline walls, 45·5× 12·8 μ, formed in clusters from free basal cells, pedicellate, pedicels hyaline, 36–65× 10–14 μ, with a central protoplasmic strand and hyaline gelatinising outer portions. Teliospores germinate *in situ*, producing apical promycelia which project beyond the surface of the leaf.

This rust closely resembles the one on *O. stylosa*, the difference being the absence of pycnia and the difference in the colour of the telia and the teliospores. These differences do not warrant the creation of a new species though the host is different. As the structure of the telia and the teliospores and the spore size do not exhibit any significant difference, this rust is also identified as *S. ascotela.*

The diagnosis of the genus *Scopella* is emended as follows:—*Pycnia* amphigenous, subcuticular and subepidermal, hemispherical or globose; *uredia* subepidermal, powdery; *urediospores* brown, pedicellate, several arising together from a cylindrical basal cell, basal cells free amongst themselves; *telia* subepidermal; *teliospores* unicellular, pedicellate many arising from a single basal cell, basal cells free amongst themselves, spore wall thin, hyaline, without germpore, teliospores often coloured, germinating *in situ*, at once by apical prolongation of the teliospore.

Scopella ascotela (Syd.) Comb. nov. Ramakrishnan and Ramakrishnan

Synonyms: *Blastospora ascotela* Syd.
Maravalia ascotela (Syd.) Mains.
Chrysocelis ascotela (Syd.) Thirumalachar.

Pycnia amphigenous mostly epiphyllous, grouped in the rust spot, subepidermal, subglobose, sunk in the tissue 155–207 × 110–125 μ; *uredia* and *æcia* not known; *telia* hypophyllous, subepidermal, clustered in the region of the spot which is thickened, waxy-yellow to golden yellow or pink; *teliospores* one celled with yellowish or orange coloured contents, clavate to cylindric, pedicellate pedicels hyaline up to 65 μ long, produced in groups

on laterally free basal cells, the spores in a group being in different stages of development, 54× 13·6 μ (37–70× 9 –18 μ) germinating *in situ* by the prolongation of the spore apex into ephemeral yellowish 4-celled promycelium, basidiospores round or oval, light yellow.

On living leaves of *Oldenlandia stylosa* O. Kze. and *Oldenlandia articularis* Gamble. Ootacamund, Nilgiris, 22–9–1946 (T. S. Ramakrishnan).

We gratefully acknowledge the help received from Dr. B. B. Mundkur, New Delhi, and from Mr. K. M. Thomas, Government Mycologist, Coimbatore, in critically reading through the manuscript and offering suggestions.

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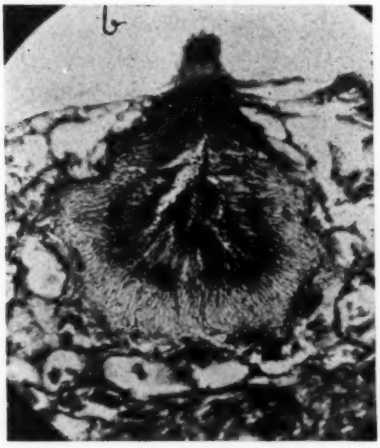
Explanation of Plate

A. One cluster of teliospores on a basal cell, (× 500).
B. Pycnium, (× 500).
C. Telia, (× 100).

a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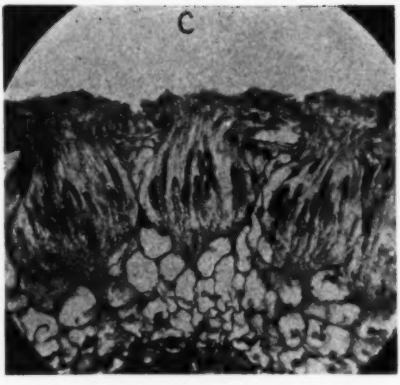
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AERATION AFFECTING GROWTH AND SPORULATION OF SOME SOIL *FUSARIA* IN LIQUID CULTURES

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Received May 2, 1947
(Communicated by Dr. T. S. Sadasivan, F.A.SC.)

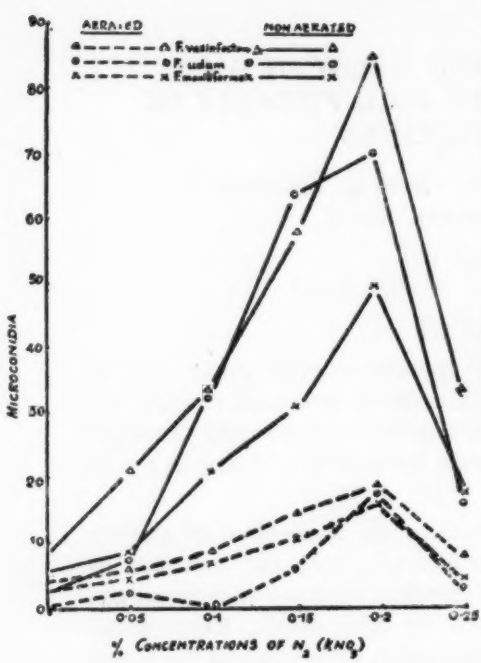
Introduction

The effect of aeration on the growth and sporulation of the genus *Fusarium* has not been studied in detail; but it is commonly recognised that aeration removes the stale gases of metabolism and ensures a pure supply of oxygen thereby accelerating the growth of a fungus both in the soil and in a pure culture medium.

Almost the first convincing evidence on the importance of aeration in the spread of soil-borne fungal infections of plants was presented by Garrett (1937). Indeed, Garrett (1936) classified *Fusarium vasinfectum* and other *Fusaria* under "diseases favoured by light soils". Experimental proof was presented by him (1936) in a decrease in growth rate of the fungus *Ophiobolus graminis* when sand was mixed with an inert substance like pure china clay; the decrease being conditioned by the decrease in soil aeration. However, evidence of a similar kind on the pure culture side had been lacking and, therefore, a study was made with *Fusarium vasinfectum*, *F. moniliforme*, and *F. udum* (isolates from cotton, paddy and pigeon-pea root-rots) by growing these fungi in aseptically aerated liquid culture solutions containing various organic and inorganic nutrients. The results have generally confirmed previous observations on the soil conditions and the occurrence of *Fusarium* root infections.

Material and Methods

The standard synthetic medium of Horne and Mitter's (glucose = 2 gm., potassium nitrate = 2 gm., potassium phosphate (tribasic) = 1·25 gm., magnesium sulphate = 0·75 gm., starch = 10 gm., distilled water=1000 ml.) was used throughout without the agar. The standard medium contained 0·028 gm. of nitrogen in the form of potassium nitrate per litre and not asparagin as the source of nitrogen. All the strains of fungi used were pure culture isolates and were type cultures received from authentic sources.



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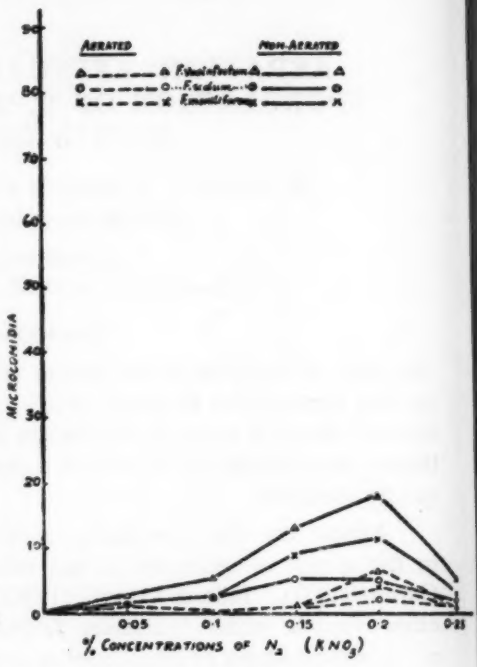

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TEXT-FIGS. 1–2.—Fig. 1. Shows micro-conidial production at varying nitrogen levels in aerated and non-aerated series in the three *Fusaria*. Fig. 2. Shows macro-conidial production at varying nitrogen levels in aerated and non-aerated series in the three *Fusaria*.

Aeration was effected by connecting in a series 250 ml. Erlenmeyer flasks, containing the sterilised liquid cultures, previously inoculated with the fungus, to an air pump. The rate of flow of the air was adjusted at 660 ml. per minute and the incoming current of air was made aseptic by passing through disinfectants contained in Woulff's flasks. The experiments were carried out at laboratory temperature, which fluctuated between 25° C.–30° C. Aeration was started twenty-four hours after inoculation; 0·1 c.c. of inoculum from liquid cultures being added to each flask. Spore numbers were determined quantitatively; the counts being taken under oil immersion by shifting the field to one division of the stage vernier in any of the four directions in a coverglass area of 7/8 sq."

For quantitatively determining the growth of the fungi, fungal mats were removed at two different periods of growth, *viz.*, 10 days and 21 days after inoculation, filtered and subsequently dried in the oven at 70°–80° C., to constant weights. They were then incinerated to determine ash weights.

Experimental

I. Effect of aeration on sporulation at various nitrogen levels.

A combined experiment was set up to find out what effect aeration and variation in total nitrogen had on sporulation. Flasks containing 50 ml. of Horne and Mitter's medium with varying concentrations of nitrogen were aerated, aeration being started 24 hours after inoculation and conducted intermittently at the rate of two running hours per day. Another series of non-aerated control flasks was maintained and cultures were examined one week after inoculation and the results are graphically presented in Text-Figs. 1 and 2.

These figures show that sporulation is optimum at 0·2% potassium nitrate concentration in both the aerated and non-aerated series. However, aeration is detrimental to optimum spore production (both micro- and macro-conidia) in any concentration of total available nitrogen.

Microconidia are prolific whereas, macroconidia are very few in comparison and among the three fungi *F. vasinfectum* shows higher micro- and macro-conidial formation than the other two species.

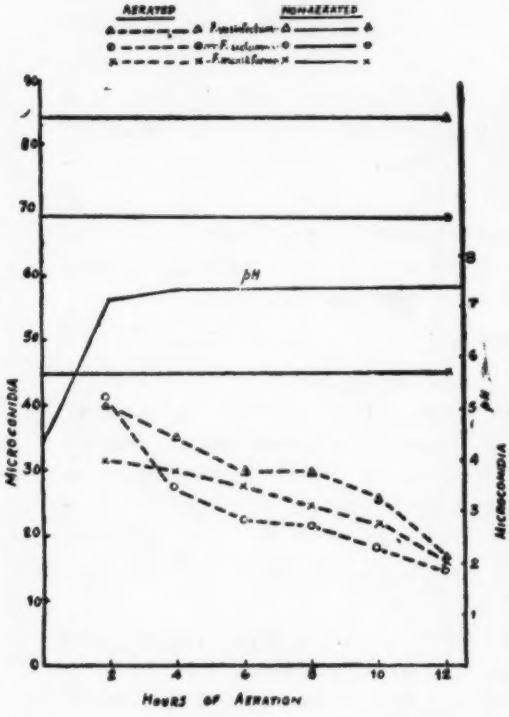
II. *Effect of aeration on sporulation and pH of culture medium.*

As root infecting fungi are in general strong aerobes, it was doubtful whether the depressing effect on sporulation could be attributed to the direct effect of aeration. It seemed more probable that continuous aeration possibly had something to do with altering the pH of the medium to an unfavourable level resulting in reduced sporulation. Therefore, examination of cultures for pH values before and after specified hours of aeration was undertaken and an experiment started to find out whether aeration altered the pH in any way.

For each species of *Fusarium* six flasks containing liquid cultures were used and connected to the air pump, thus having three series, of six flasks for each fungus, with similar numbers for the non-aerated control. Initial pH values were taken prior to aeration for both the series. All the 3 series were aerated for two hours, one flask from each series being detached and pH values taken for the three fungi whilst determining at the same time pH values for the control series. It must be mentioned here that aseptic aeration for 2, 4, 6, 8, 10 and 12 hours of uninoculated media does not in any way alter the pH as compared with the non-aerated series. However, with fungus inoculum growing, the aerated series did show rise in pH values but this rise from the acidic side, *i.e.*, pH 4·4 to pH 7·4 was similar both in aerated and non-aerated series. Along with final pH readings which were

taken on the seventh day after detachment for aerated cultures, spore counts were made. Control series were also examined for spore numbers. This evidence tended to show that duration of aeration had no direct effect on the pH of the media but still produced profound changes in the number of micro- and macro-conidia produced, which effect is attributable, therefore, directly to aeration.

The results are graphically presented in Text-Figs. 3 and 4.



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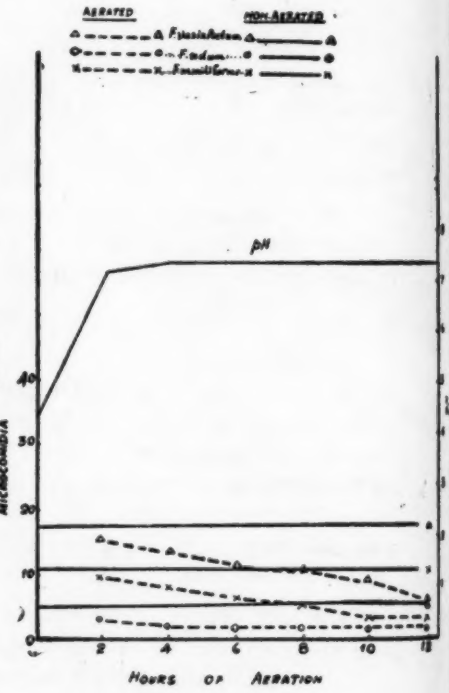

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TEXT-FIGS. 3–4.—Fig. 3. Shows micro-conidial production by the three *Fusaria* at different hours of aeration. Changes in pH are also presented. Fig. 4. Shows macro-conidial production by the three *Fusaria* at different hours of aeration. Changes in pH are also presented.

Text-Figs. 3 and 4 show that

1. With increase in number of hours of aeration, spore production is on the decline.

2. Percentage fall in sporulation on two hours aeration is highest in *F. vasinfectum*, followed by *F. udum* and lowest in *F. moniliforme*.

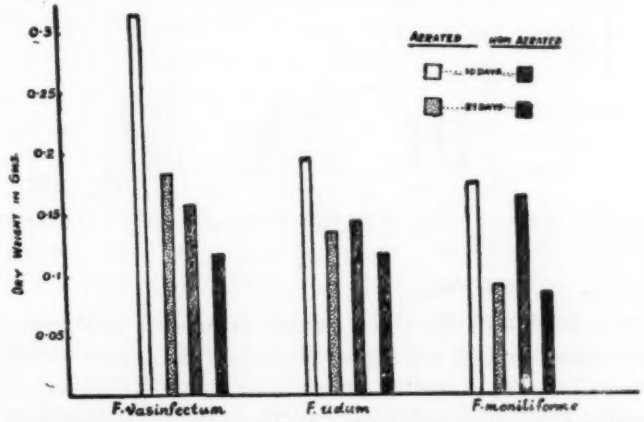
3. Macro-conidia as usual are fewer in number than micro-conidia which appears as a uniform characteristic in all the three species.

4. Maximum aeration brings about a marked fall in all the three cases.

5. Duration of aeration has no effect on the pH of the culture media.

III. *Effect of aeration on weight of fungus.*

The fall in micro-conidial and macro-conidial numbers noticed in Experiment I under aerated conditions in liquid media containing varying amounts of nitrogen necessitated the study of the behaviour of the mycelia of the three species of *Fusarium* from the quantitative point of view. It has been shown recently in this laboratory by one of the authors (Yogeswari, unpublished) that variation in the dry and ash weights of *Fusaria* growing in liquid cultures with different nutritive substrates, can be very accurately determined quantitatively, with suitable replications within treatments. Thus, it was noticed that aeration of the liquid cultures considerably increased both dry and ash weights of the mycelial mats in all the three species of *Fusarium* under investigation. The details of the results of this experiment are presented diagrammatically in Text-Figs. 5 and 6.



TEXT-FIG. 5. Shows dry weights of the three *Fusaria* after 10 days and 21 days growth in aerated and non-aerated cultures.

Text-Fig. 5 shows that

1. Dry weights of the three *Fusarium* spp. in aerated and non-aerated cultures taken after ten days are higher than those determined after twenty-one days,

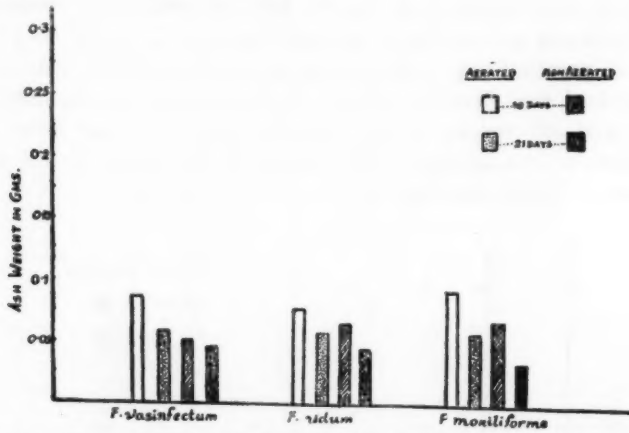
2. (*a*) *F. vasinfectum* in both ten-days old and twenty-one days old aerated cultures shows better growth over the other two fungi.

(*b*) *F. moniliforme* shows lowest weight.

(*c*) In non-aerated ten-day old cultures *F. moniliforme* shows a slight increase in dry weight over *F. vasinfectum* and *F. udum*.

(*d*) In non-aerated twenty-one day old cultures fall in *F. moniliforme* is very marked being lower than *F. vasinfectum* and *F. udum* dry weights.

3. Difference in dry weights between ten and twenty-one day old aerated cultures of the three *Fusaria* is very pronounced in *F. vasinfectum* while in non-aerated cultures, difference in dry weights is most marked in *F. moniliforme*.



TEXT-FIG. 6. Shows ash weight of the three *Fusaria* after 10 days and 21 days growth in aerated and non-aerated cultures.

Text-Fig. 6 shows that

1. Ash weights of the three *Fusaria* in aerated and non-aerated cultures taken after ten days are higher than those taken after twenty-one days but unlike in Text-Fig. 5, all the strains show almost level weights in ten-day old cultures. The fall in weight after twenty-one days is highest in *F. moniliforme* in both the series.

2. Fall in the ash weights after twenty-one days growth is not so great as compared with the dry weights.

DISCUSSION

Aeration of liquid culture media under the present experimental conditions retarded the spore producing ability of *F. vasinfectum*, *F. moniliforme*

and *F. udum*. On the other hand, very distinct increase in dry and ash weights of the three species was observed under similar conditions of aeration over their non-aerated controls. It was felt at the time when these results were obtained that aeration of liquid cultures possibly introduced some changes in the reaction of the medium, which in turn resulted in the inhibition of the spore producing ability followed by an increase in mycelial mat formation. But further experiments showed that the pH of both aerated and non-aerated series ran parallel with each other, although shifting the reaction from the acidic to alkaline in both cases. It was further thought that changes in the availability of total nitrogen possibly governed spore production. But even here it was discovered that increase or decrease in total nitrogen did, doubtless, bring about changes in the quantity of spore produced, but the general spore-producing ability of the fungi was still very low in the aerated as compared with the non-aerated series. These experiments have brought to light the functions of aeration in increasing vegetative growth of *Fusaria* as well as in delimiting their ability to sporulate. Valuable comparisons can be made with already established facts that light sandy soils promote ramification of mycelia induced by conditions of abundant soil aeration and it is significant to note that the more rapid disappearance of various soil fungi in light soils is possibly due to the poor spore-forming tendency of the fungi concerned. Further work on soil isolations from cotton-growing tracts with light soils (which is in progress) may confirm the finding which has been conducted under pure culture.

That saprophytic fungi are most active in the decomposition of plant residues under conditions of abundant soil aeration is an established fact (Waksman, 1931; Garrett, 1938, 1939). The purport of this paper is mainly to emphasize that these findings on the behaviour of soil *Fusaria* in pure culture, both aerated and non-aerated, is in keeping with established facts that disappearance of various soil fungi is most rapid in loose soils possibly due to their inability to sporulate normally. The vegetative growth under such aerated conditions although better than non-aerated does not contribute towards the longevity of the fungus, since the inevitable micro-biological antagonism shortens the vegetative phase of fungal activity more easily than when confronted with prolific spore development, the latter being less vulnerable to micro-biological attack.

Summary

1. Effect of aseptic aeration on growth and sporulation of the three soil fungi, *viz.*, *F. vasinfectum*, *F. moniliforme*, and *F. udum* was studied in detail.

2. Sporulation of *F. vasinfectum*, *F. moniliforme* and *F. udum* was optimum at 0·2% nitrate nitrogen in standard Horne and Mitter's liquid medium.

3. Aeration stimulated mycelial growth (on both dry and ash weight basis) but inhibited sporulation (quantitatively determined).

4. Aeration had no direct effect on the pH of the culture medium.

5. Sporulation decreased with increasing hours of aeration.

ACKNOWLEDGMENT

We are greatly indebted to Dr. T. S. Sadasivan, for his helpful criticism and guidance, in the course of this investigation.

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FUSARIUM SP. PARASITIC ON *EPIPYROPS*, A LEPIDOPTEROUS PARASITE OF THE SUGARCANE *PYRILLA*

By S. Y. Padmanabhan

(*Sugarcane Research Station, Anakapalle*)

Received February 14, 1947

(Communicated by Prof. L. Narayana Rao, F.A.SC.)

Introduction

In October 1939, the late Dr. John Muliyal brought to the writer's notice pupæ of *Epipyrops* sp. attacked by a fungus. In its larval stage, *Epiopyrops* is a parasite on *Pyrilla* the well-known pest of sugarcane. A study was undertaken to identify the fungus and to establish its pathogenicity on *Epipyrops*.

Description of the Material

The pupæ are rectangular in shape, 22–25 mm. long and 8–9 mm. broad and were attached to the ventral surface of the sugarcane leaf, the longer axes of the pupæ being along the length of the leaf. The pupæ were covered over by salmon coloured mycelium. When a portion of the mycelium was examined under the microscope innumerable conidia of *Fusarium* sp. were observed.

Identification Tests

The morphology of the fungus was studied in detail. The characters of the conidia produced on the mycelium parasitising *Epipyrops* were compared with those produced by single spore cultures isolated from the former. A remarkable variability was at once perceived in the spores produced in culture. One of the single spore isolates differed from the fungus obtained directly from *Epipyrops* in the total absence of macro-conidia, while both the micro- and macro-conidia were present in other isolates.

Three isolates were used in the study of the morphology of the fungus: (i) the fungus from *Epipyrops*, (ii) a single conidial culture from (i) but producing only microconidia, (iii) a single conidial culture from growths isolated from artificially infected *Epipyrops* pupæ [the healthy *Epipyrops* pupæ were artificially infected in the laboratory with spore suspensions of (ii) above].

The two isolates, (ii) and (iii) were grown on the following seven media:

1. Potato cylinders.
2. 2% potato Dextrose agar.
3. 5% potato Dextrose agar (Wollenweber, *et al.*, 1925).
4. Oatmeal agar.
5. Steamed rice (Wollenweber, *et al.*, 1925).
6. Brown's standard agar (Brown, 1925).
7. Brown's starch agar (Brown, 1925).

The potato cylinders were prepared in the usual manner and were sterilized at 10 lbs. pressure for 45 minutes.

The oatmeal agar was prepared by taking 100 grams of Quaker Oats in water, using a sufficient quantity of water to bring the oats into solution when warmed. The warm solution was strained through cheese cloth, taking care that no crushing or pressure was applied to help the slow streaming through of the solution. The solution was made up to 1000 c.c., tubed, and sterilized at 10 lbs. pressure for 45 minutes.

Triplicate tubes of the cultures grown in the seven media were maintained at two temperatures, 18–20° C. and 35° C., to study the range of variation exhibited by the fungus, and to identify it on the basis of the characters so observed taken in conjunction with the morphology of the fungus from the naturally occurring parasite on *Epipyrops*. For convenience, the two cultures will be referred to in the following text as culture 1, and culture 2, while the *Fusarium* sp. from *Epipyrops* will connote the fungus obtained directly from the parasitised material.

Observations were made on the 10th and 21st day on the amount and colour of aerial mycelium and substrate, production of sclerotia stroma and pionnotes. The data are recorded in Tables I and II.

The spore measurements and data on the percentage occurrence of the different septate spores were obtained on the 22nd day in culture 1, and on the 23rd and 24th days in culture 2. The spore measurements, etc., were made only on the cultures maintained at 18° C. The data are presented in Tables III, IV, V, VI. The spore measurements data for *Fusarium* sp. from *Epipyrops* are shown in Table VII.

The data presented in the tables may be summarised as follows: In both the cultures, the aerial mycelium is colourless or white.

TABLE I

Showing the amount of Aerial Mycelium, colour of Aerial Mycelium and of Substrate, Conidial Production, Selerotia, Stroma, Ponnotes on the 10th and 21st days of growth at two Temperatures in different media

Medium	Culture	Temperature series O.C.	Aerial Mycelium		Surface of substratum		Selerotea Stroma	Pinnoter
			10th day	21st day	10th day	21st day		
Potato cylinder	I	18	White	White abundant, 0·3 septate spores, abundant	Unchanged	Olive gray	Absent	Absent
		35	White	White; abundant, 0·2 septate spores abundant	Dark Olive gray	Deep Olive buff; iron gray	Absent	Absent
	II	18	White	White; abundant conidia 0·5 septate abundant	Light vinaceous Cinnamon	Buff-pink, deep colonial buff; spores scattered over the surface	Absent	Absent
		35	White	White abundant	Apricot buff	Apricot buff; spores scattered over the surface	Absent	Absent
2% Potato Dextrose	I	18	White	White and deep dull bluish violet; moderate abundant; conidia 0·1 septate, abundant	Pale violet gray	Deep slaty brown; conidia scattered, 0·1 septate	Absent	Thin
		35	Lacking	Lacking	Onion skin pink dark nigrosin violet	Cinnamon buff, dark nigrosin violet; conidia 0·1 septate, scattered	Absent	Thin
	II	18	White	White scanty; conidia 0·6 septate	Salmon buff	Salmon buff, conidia 0·6 Septate	Absent	Thin
		35	Lacking	White scanty conidia few; 0·3 septate mostiy	Flesh ochre	Flesh ochre; abundant conidia 0·5 septate	Absent	Thin

TABLE I—(*Contd.*)

Medium	Culture	Temperature series 0°C.	Aerial Mycelium		Surface of substratum		Sclerotea Stroma	Pinnoter
			10th day	21st day	10th day	21st day		
5% Potato Dextrose	I	18	White	White; moderately abundant as in 27	Unchanged	Deep slaty brown	Absent	Thin
		35	Lacking	Lacking	Dark nigrosin violet; pinkish Cinnamon	Dark nigrosin violet and buff pink; conidia scattered, 0·1 septate	Absent	Thin
	II	18	White	White	Salmon buff	Salmon buff	Absent	Thin
		35	Lacking	White; scanty conidia as in 2%	Flesh ochre	Flesh ochre	Absent	Thin
Oatmeal Agar	I	18	White	Lacking	Unchanged	Hyssop violet; argyle purple. Conidia 0·1 septate	Absent	Thin
		35	White trace of spinal red	Lacking	Unchanged	Unchanged; Conidia abundantly 0·1 septate	Absent	Thin
	II	18	White	White moderately abundant; conidia abundant; 0·7 septate	Salmon colour	Apricot buff Conidia scattered	Absent	Thin
		35	Lacking	White; abundant; conidia abundant, mostly 0·3 septate	Salmon buff	Salmon buff, Conidia scattered	Absent	Thin
	I	18	White	White abundant; conidia 0·2 septate	Cameo pink	Thulite pink	Absent	Absent
		35	White	White; abundant 0·2 septate abundant	Coral pink and Apricot orange	Coral pink ocheroceous orange, wax yellow	Absent	Absent

TABLE I—(*Contd.*)

Medium	Culture	Temperature series 0°C.	Aerial Mycelium		Surface of substratum		Sclerotea Stroma	Pinnoter
			10th day	21st day	10th day	21st day		
Steamed Rice	II	18	White	White abundant; conidia abundant 0·5 celled; mostly 0·5 septate	Salmon colour deep dark olive buff	Conidida thinly scattered	Absent	Absent
		35	White	White scanty (Bacterial contamination) conidia mostly 2·3 septate	Fresh ochre	Apricot buff, cream buff, orange at the bottom; Carnelian red at the top and Cinnamon buff	Absent Conidia abundant on the surface	Absent
Brown's Standard Agar	I	18	White	White scanty; conidia abundant; septate, mostly 2·3 septate	Unchanged	Unchanged, conidia scattered, 0·1 septate	Absent	Absent
		35	Lacking	Lacking	White	White, conidia, abundant septate; 0·3 septate	Absent	Absent
	II	18	White	White; scanty; conidia 0·5 septate, abundant	Lacking in colour	Lacking in colour, conidia thinly scattered	Absent	Absent
		35	Lacking	Lacking	Apricot buff	Apricot buff; abundant conidia. mostly 0·3 septate	Absent	Absent
Brown's Starch Agar	I	18	Lacking	Lacking	Unchanged	Lumsere blue; abundant conidia 1·2 septate	Absent	Thick
		85	Lacking	Lacking	Prussian blue	Dull purplish black; abundant conidia 1·2 septate	Absent	Absent
	II	18	White	White scanty conidia 0·5	Pale Salmon	Salmon; conidia, thinly scattered on the surface	Absent	Absent
		35	White	White scanty; conidia mostly 0·3 septate	Apricot orange	Apricot buff; conidia abundant	Absent	Absent

TABLE II

Showing the form of Conidia and Chlamydospores observed on the 10th and 21st days of growth at two different Temperatures on Seven Different Medium

(5% Pot. dextrose, not shown as the data were similar to those in 2% potato dextrose.)

Medium	Culture	Temperature series 0°C.	Conidia in aerial mycelium	Conidia on the stromatal layer	Chlamydospores
Potato cylinder	I	18°C.	Single, continuous and 1·3 septate; septate spores comparatively abundant; ovoid to spindle shaped, cylindrical, slightly curved; sometimes slightly vaculate, apex rounded		Absent
	I	35°C.	Single,, continuous, 1·3 septate. spores ovoid, spindle-shaped; cylindrical or slightly curved hyaline, abundant		do
	II	18°C.	Single, continuous, 1·5 septate; mostly 3 and 5 septate; ovoid to cylindrical microconidia; macro conidia straight or rarely curved; bluntly pointed ends; without a foot-cell	Spores scattered over the surface; surface slightly slimy	do
	II	35°C.	Single, continuous, predominantly septate; 1·5 septate mostly 3 septate; microconidia ovoid to cylindrical or curved; micro-conidia sickle-shaped, spindle-shaped cylindrical, straight with blunt ends slightly foot celled base, curved spores with a fine curved point	Spores abundant on the slimy surface	do
Potato Dextrose	I	18°C.	Single, continuous (1·2 septate spores also seen) ovoid to spindle-shaped hyaline, abundant	Thinly scattered on agar surface predominantly continuous; ovoid to spindle-shaped; occasionally 1·3 septate; thin straight or slightly curved and rounded apex and bluntly pointed or slightly foot-celled base; separations indistinct, hyaline	do
	I	35°C.	do	do	do
	II	18°C.	Single, continuous and 1 septate; microconidia ovoid to spindle-shaped or straight cylindrical; miacroconidia, curved, straight or slightly curved with bluntly pointed ends or finely pointed ends	Spores thinly scattered on the agar surface, rarely with slightly foot-celled base	do

TABLE II—(*Contd.*)

Medium	Culture	Temperature series 0°C.	Conidia in aerial mycelium	Conidia on the stromatal layer	Chlamydospores
Potato Dextrose	II	35°C.	Predominantly septate; straight with blunt ends; curved; slightly foot-celled base; mostly 0·3 septate; rarely 4·15 septate	Abundantly scattered over the surface; ovoid microconidia typically curved and straight 0·5 septate, frequently curved ends blunt or bluntly pointed With or without a foot-celled base; indistinctly septate	Absent
Oatmeal Agar	I	18°C.	Single, continuous, rarely septate; ovoid cylindrical spindle-shaped; rounded apex and base; not abundant	Thinly scattered on Agar surface. mostly continuous rarely 1 septate; ovoid; cylindrical spindle-shaped rounded apex, base blunt without any foot cell; conidia comparatively not abundant	do
	I	35°C.	Single continuous rarely 1·2 septate spores; ovoid cylindrical spindle. shaped hyaline, abundant	Thickly scattered on agar surface- spindle-shaped; occasionally 1·3 septate straight, slightly curved apex blunt or rounded; septations indistinct; hyaline	do
	II	18°C.	Conidia 0·7 septate; mostly septate spores; 3·5 septate spindle-shaped elongated slightly pointed ends, curved, slightly foot-celled micro-conidia ovoid cylindrical rounded apex	Thinly scattered on the surface	do
	II	35°C.	Conidia 0·5 septate; mostly 0·7 septate ovoid to spindle-shaped, stout. thick and straight or narrow and slightly curved the ends blunt or finely pointed; hyaline, occasionally vaculate	Thinly scattered on the surface	do
Steamed Rice	I	18°C	Single continuous, mostly occasionally 1 septate, rarely 2 septate, ovoid to spindle-shaped curved, straight cylindrical apex rounded; blunt abundant	Spores thinly scattered on the surface	do
	I	35°C.	do	do	do
	II	18°C.	Conidia 0·5 celled. mostly 3·5 celled spindle-shaped, straight or slightly curved ends blunt or bluntly pointed, hyaline; occasionally vaculate	Spores thinly scattered on the surface	do
	II	35°C.	Conidia very irregularly shaped, mostly 0·3 septate; rarely 4·5 septate, spherical to ovoid and spindle-shaped with blunt ends	Abundantly scattered on the surface	do

TABLE II—(*Contd.*)

Medium	Culture	Temperature series 0° C.	Conidia aerial mycelium	Conidia on the stromatal layer	Chlamydospores
	II	18°C.	Conidia very thin walled, 0·5 septate ovoid to spindle shaped, blunt or bluntly pointed ends	Conidia thinly scattered on the surface	Absent
		35°C.	Conidia 0·5 septate, mostly 0·3 ovoid to cylindrical, spindle-shaped, blunt or bluntly pointed	Conidia abundantly scattered over the surface	do
Brown's Starch agar	I	18°C.	Single continuous, very rarely 1 septate ovoid to spindle-shaped occasionally curved, apex blunt abunddant with or without foot-celled base		do
		35°C.	Single or false heads ovoid to cylindrical 1·2 septate straight and slightly curved, apex rounded, without foot-cells, abundant hyaline		do
	I	18°C.	Single predominantly septate, 0·3 septate; mostly straight ovoid to spindle-shaped, occasionally slightly curved apex rounded or pointed very distinct septate, abundant	Single, continuous 1 septate occasionally 2 septate; spindle-shaped ovoid, rarely curved, apex rounded or pointed, septate distinct; abundant	do
		35°C.		Septate spores comparatively abundant; 1·3 septate ovoid, cylindrical furoid, curved to sickle-shaped apex blunt or bluntly pointed, base slightly foot-celled	do
Brown's Standard Agar	II	18°C.	Conidia thin-walled 0·5 septate; microconidia ovoid to spindle-shaped, septate spores curved with blunt or bluntly pointed ends	Spores thinly scattered on the surface	do
		35°C.		Single continuous or septate; 0·3 septate ovoid to cylindrical or spindle-shaped blunt to pointed ends, curved to sickle-shaped without foot cell–conidia typically like those of I in 18°C. and 35°C.	do

TABLE III

Showing the Percentage Occurrence of the different septate spores in the Seven Media of Culture I at 18° *C. (after* 23 *days)*

Medium	0 septate	1 septate	2 septate	3 septate
Potato cylinder ..	96	4	..	..
2% P.D. Agar ..	98	2	trace	trace
5% P.D. Agar ..	..	..	..	..
Oatmeal ..	98	2	..	..
Steamed rice, medium ..	98	2	trace	..
Brown's starch, medium ..	98	2	..	..
Brown's standard medium ..	98	2	..	..

TABLE IV

Showing the mean measurements in μ *of length, the range in length of the Conidia of Culture I at* 18° *C. after* 23 *days*

Medium	0 septate		1 septate		2 septate	3 septate
	Mean	Range	Mean	Range		
Potato cylinder	8·94	4–16	13·52	10–20		
P.D.A. 2% ..	7·28	4–12	15·86	8–24	18·4	32
Oatmeal ..	8·68	4–16	14·6	10–24	22·8	20
B.A. ..	8·4	4–16	18·0	12–24		
B.St. A. ..	6·4	2–12	13·52	10–20		
Rice ..	8·84	4–16	15·72	10–24	19	24
	8·1	2–16	15·4	8–24	20·1	25·3

TABLE V

Spore measurements of Culture II from larva of Epipyrops taken from twenty-three days old cultures kept at 18–20° C.

	Medium	0 septate		1 septate		2 septate		3 septate		4 septate		5 septate		6 septate		7 septate	
		L	B	L	B	L	B	L	B	L	B	L	B	L	B	L	B
Mean measurement ..	2% Dextrose Agar	11·2	3·6	13·68	3·96	36·82	3·96	32·76	3·96	36·36	3·96	37·26	4·3	7			
Range ..		10·8 to 11·6	3·24 to 3·96	10·8 to 18·0	3·6 to 4·68	14·4 to 18·72	3·24 to 4·68	15·2 to 34·20	3·6 to 5·76	25·92 to 39·6	3·6 to 5·76	32·4 to 44·28	3·6 to 6·84				
Percentage occurrence ..		4%		7%		2%		63%		3%		21%					
Mean measurement ..	Potato cylinder	8·64	3·3	12·96	4·3	18·0	4·3	25·42	4·3	29·66	4·68	39·74	4·68	45·9	5·4	52·2	5·
Range ..		4·68 to 14·4	2·5 to 3·6	7·2 to 16·2	3·24 to 6·12	12·6 to 21·6	3·6 to 4·96	18·72 to 35·28	3·24 to 6·48	28·8 to 39·6	3·6 to 6·48	30·6 to 52·2	3·6 to 6·48	30·6 to 52·2	5·4	52·2	5·
Percentage occurrence ..		3·2%		7·6%		12·8%		66·4%		6%		3·2%		0·8%			
Mean measurement ..	Oatmeal	9·0	3·24	13·5	3·96	30·2	3·96	24·5	3·96	30·096	3·96	35·64	3·96	45·72	4·32		
Range ..		7·2 to 10·8	2·88 to 3·6	12·6 so 14·4	2·88 to 4·68	17·64 to 25·2	3·24 to 4·32	36·36 to 17·28	2·88 to 4·32	19·8 to 38·88	2·88 to 4·68	23·04 to 51·12	3·6 to 4·68	45·72 to	4·32 to		
Percentage occurrence ..		2·7%		3·6%		5·8%		47·3%		13·3%		24·6%		2·7%			
Mean measurement ..	Steamed rice	9·36	3·96	15·12	44·6	16·2	3·6	26·06	4·3	34·06	4·3	43·91	4·3				
Range ..		8·28 to 10·14	3·6 to 4·32	14·4 to 18·0	3·6 to 5·4	16·2 to ..	3·6 to ..	18·72 to 35·64	3·6 to 4·68	18·8 to 43·92	3·96 to 4·68	32·4 to 61·2	3·6 to 4·68				
Percentage occurrence ..		6·5%		9·5%		5·5%		44·2%		6·8%		27·5%					
Mean measurement ..	Brown's standard Agar	10·188	3·456	15·84	3·53	19·8	3·5	27·27	3·96	33·92	3·96	36·31	3·96	45·54	4·3		
Range ..		10·08 to 12·6	3·24 to 3·6	11·52 to 21·6	3·24 to 3·6	16·2 to 23·4	3·24 to 3·6	18·03 to 35·28	3·6 to 4·68	33·1 to 36	3·6 to 5·04	27·36 to 43·2	3·6 to 4·68	45 to 46·5	3·6 to 5·04		
Percentage occurrence		·46%		4·1%		9·1%		39·5%		10·9%		34·1%		1·8%			

TABLE VI

Mean measurement and percentage occurrence of Septate Spores in Culture II

Septation	0	1	2	3	4	5	6	7
Percentage ..	3%	6%	7%	52%	8%	23%	1%	Trace
Mean length ..	9·59	14·25	18·2	27·2	28·82	38·38	45·72	52·2
Total Range ..	4·68 to 14·4	7·2 to 21·6	12·6 to 25·2	15·2 to 36·36	19·8 to 43·92	23·4 to 52·2	45·7 to 45·92	52·2
Mean Breadth ..	3·59	4·0	3·79	4·0 (4·09)	4·0 (4·09)	4·0 (4·21)	5·0 (4·68)	5·4
Total Range ..	2·5 to 3·96	2·88 to 6·1	3·2 to 4·9	3·2 to 6·48	2·88 to 6·48	3·6 to 6·8	4·32 to 6·8	5·4

TABLE VII

Measurement of spores taken directly from the diseased pupæ of Epipyrops

Septation of spores		0	1	2	3	4	5	6	7
1	Mean L	14·56	21·36	26·0	31·6	37·8	43·2	44	
2	Range in L	10–16	14–30·0	16–32	16–44	32–44	34–52	34–52	
3 Measurement of spores in μ	Mean B	4·0	4·0	4·4	4·5	4·5	5·0	5·0	
4	Range in B	2·8 4·2	2·8 4·2	3·0 6·2	3·0 6·4	3·0 6·4	3·8 7·0	3·8 7·0	
Percentage occurrence of spores		14·6	21·5	14·6	34	6·7	6·5	1·3	0·6

TABLE VIII

Table comparing the percentage Occurrence of septate spores, Mean length, Mean Range in length of spores of Fusarium *sp. from* Epipyrops *and the Cultures*, 1, 2 *and* Fusarium moniliforme *var. subglutinans*

Septation	0 Sep	1 Sep	2 Sep	3 Sep	4 Sep	5 Sep	6 Sep	7 Sep
(A) Percentage occurrence								
1 Spores from diseased larva ..	14·6%	21·5%	14·6%	34%	6·7%	6·7%	1·3%	0·6%
2 Spores from Culture I ..	97·7%	2·3%	Trace	Trace	..	..	..	..
3 Spores from Culture II ..	3%	6%	7%	52%	8%	23%	1%	Trace
(B) Mean length in μ								
1 Spores from diseased larva ..	14·56	21·36	26·0	31·6	37·8	43·2	44	..
2 Spores from Culture 1 ..	8·1	15·41	20·1	25·3	..	..	..	..
3 Spores from Culture II ..	9·59	14·25	18·2	27·2	28·2	38·8	45·72	52
4 *F. monoliforme* Sheld. var. *subglutinans* ..	9	17	..	32	..	50	..	..
(C) Mean range in length in μ								
1 Spores from diseased larva ..	10–16	14–30	16·32	16–44	32–44	34–52	34–52	
2 Spores from Culture I ..	2–16	8–24	19–23	20–32	..	..	..	
3 Spores from Culture II ..	5–14	7–22	13–25	15 36	20–44	23–52	45–52	
4 *F. monoliforme* Sheld var. *subglutinans*	7–12	14–20	..	25–48	..	43–53	..	

The stroma is purple or pink in culture 1 (Ridgeway, 1912), while it is uniformly salmon or salmon buff in culture 2. The cultures agree closely with each other regarding the production and the characters of the micro-conidia. They are found generally thinly scattered on the agar surface or occasionally grouped together in false heads, predominantly continuous ovoid to spindle shaped; occasionally 1–3 septate, thin, straight or slightly curved with rounded apex, with bluntly pointed or slightly foot-celled base; the septations are indistinct and hyaline (Text-fig. B).

Abundant conidia are found in the aerial mycelium. The conidia are not produced in chains.

In addition to possessing micro-conidia as above described, culture 2 is also characterised by the production of predominantly septate spores. The spores produced in aerial mycelium are 0–5 septate, rarely 6–7 septate, rod to spindle-shaped, with blunt ends or ends tapering to a blunt point, or with the ends slightly curving and tapering, without foot-celled base or rarely with distinct or indistinct foot-cell (Text-fig. C).

[Figs. A–C.—Fig. A. Conidia of *Fusarium moniliforme* Sheld. var. *subglutinans*. *Wrand Rkg.* from pupa of *Epipyrops* (× 400). Fig. B. Conidia of above from culture 1, producing only microconidia (× 600). Fig. C. Conidia of above from culture 2, producing conidia as in nature (× 400).

Chlamydospores are absent in both the cultures.

The conidia from the mycelium of the parasitised *Epipyrops* are closely similar to those described under culture 2 (Text-fig. A).

Taxonomy

According to Wollenweber and Reinking's key for the identification for the groups and sub-groups of the genus *Fusarium* the abundant micro-conidia and lack of macro-conidia in aerial mycelium and of chlamydospores, and nature of colours, place culture 1, in " Liseola ".

Micro-conidia are not in chains. Blue sclerotia are absent. If the absence of blue sclerotia is recognized as a variable characteristic as Wollenweber (1935) does in the description of *F. moniliforme* Sheldon, then the other characters closely approximate to the above fungus. Further, the absence of conidia in fast chains brings it to *F. moniliforme* (Sheldon) var. *subglutinans* Wr. and Rkg. If the production of blue sclerotia is given more importance in distinguishing species, the fungus is brought down to *F. neoceras* Wr. et. Rkg. But the spores are not as long as those of *F. neoceras*, being little more in fact than half the length. Regarding culture 2, and the fungus from parasitised *Epipyrops* the spores in the aerial mycelium are not those of " Liseola ".

The spore characters place the latter in the section, " Lateritum " which is after all very close to " Liseola " and overlaps it as seen in the Key of Wollenweber (also Padwick, 1941). The key characters employed to distinguish the two sections is the production of micro-conidia in chains in the latter and their being not in chains in the former. In *F. moniliforme* v. *subglutinans* the conidia are not in chains, but the fungus belongs to " Liseola " and is described under the section by Wollenweber.

The presence of septate spores instead of microconidia raises an important issue. Wollenweber (1935) states that, " *F. moniliforme* is variable and occurs in forms which oscillate in the septation of the conidia, sometimes suddenly rising to develop highly septate sickle-shaped spores in sporodochia and pionnotes far surpassing the normal in number, then further relapsing to produce mostly the micro-conidia ". Further, according to Subramaniyam and Chona (1938) the fungus isolated from sugarcane suffering from ' wilt ' in Bihar and identified as *Chephalsporium sacchari* Butler by Mcræ produced abundant macro-conidia typically like those of *F. moniliforme* in Holland. When the cultures were received in India, they produced only microconidia. Thus the variability in spore production of this fungus recorded in this paper is in conformity with the earlier observations mentioned above.

In comparing the data presented in Tables I and II, it will be seen that the two cultures agree closely with each other in the amount of growth, colour and amount of conidial production in all media except oatmeal and in the remarkable similarity in appearance and measurements of the 0–3 septate spores in Oatmeal, rice, and Brown's standard agar. In the above three media culture 2, produced mostly 0–3 septate spores.

Thus the fungus isolated from *Epipyrops* is identified as *F. moniliforme* Sheld. var. *subglutinans* Wr. and Rkg. [*Gibberella Fujikuori* (Sawada) Wr. var. *subglutinans* Edwards].

Parasitism of F. moniliforme *var.* subglutinans *on* Epipyrops

Experiment I.—Ten adults and nymphs of *Pyrilla* with *Epipyrops* larvæ were collected from the field, preserved in wire gauze chambers and fed on fresh sugarcane leaves. Using a small atomiser the parasites along with the hosts were sprayed upon with spore suspension from a fresh 20 days old culture of *F. moniliforme* var. *subglutinans*. The larvæ were not affected in any way even at the end of a week.

Experiment II.—Specimens of pupæ attached to the sugarcane leaves were collected and sprayed upon with spore suspension of the above fungus as in experiment I. A profuse salmon coloured mycelium developed on the pupæ within 48 hours. The fungus was re-isolated and identified as *F. moniliforme* var. *subglutinans*.

The fungus is a parasite of *Epipyrops* in its pupal stage only. It is not able to parasitise the larva. In nature also only pupæ have been found attacked by the fungus.

Summary

1. A species of *Fusarium* was found parasitising pupæ of *Epipyrops* which in its larval stage is a parasite on *Pyrilla*, a pest of sugarcane.

2. A remarkable variability between the isolates of the fungus was noticed. One set of isolates produced only microconidia in culture, while the rest of the cultures produced both micro- and macro-conidia. In the natural state both the micro- and macro-conidia were present.

3. The morphological features are given in detail.

4. The conidial character bring the culture producing only the micro-conidia nearest to *F. moniliforme* (Sheld.) var. *subglutinans* but blue sclerotia, however, are absent. But as this is stated to be a variable character in literature the fungus is regarded as *Fusarium moniliforme* var. *subglutinans*.

ACKNOWLEDGEMENTS

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STUDIES ON SCLEROTIUM-FORMING FUNGI

I. *Sclerotium cepivorum* Berk and *S. tuliparum* Klebahn

Part 1. Cultural Studies

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Introduction

A number of workers have attempted to correlate restriction of parasitic fungi to particular host plants with certain biochemical relationships, for example, with toxic properties of plant juice and with the capacity of fungi to secrete the pectinase enzyme. The primary object of the investigations in this series is to explore the position with regard to certain sclerotium-forming fungi. In the first instance the fungi selected, *Sclerotium cepivorum* and *Sclerotium tuliparum*, were chosen as being similar in habitat, in the type of plant part attacked, and to some extent at least in morphological features. The first step in the investigation was to determine how far these two fungi were restricted to their particular hosts, onion and tulip, respectively. That being established, the problem was then to try to explain the basis of the specialisation shown.

While the central idea was as outlined above, the investigation naturally followed a variety of lines, leading to a detailed cultural study of the two organisms concerned.

Historical

The literature dealing with the fungi *Sclerotium tuliparum* Klebahn and *Sclerotium cepivorum* Berk is rather extensive. In the following account each fungus will be treated separately, in the order named.

The bulb-rot of tulips was long known in Holland and Germany where it caused severe damage. It was reported from Holland at least as early as 1884 and Wakker[41] appears to have been the first to describe the disease which he designated merely as the "tulpenziekte". He has given extraordinarily clear and accurate symptoms of the disease and the characters of the pathogen.

Ritzema Bos[35,36] describes a disease which was very destructive at the time in certain parts of Holland and which from the symptoms given was obviously the gray bulb rot. However, there was a certain amount of

confusion in his account with the blight due to *Botrytis* (" fire "). He states that diseases of Iris, Hyacinth and Gladiolus due to the same *Sclerotium* also occur.

Klebahn[20,21,22] in his earlier papers made the same confusion between two tulip diseases but later recognised them as distinct. He is responsible for the name *Sclerotium tuliparum*, and he showed that the same fungus attacks a large number of hosts, in particular *Iris hispanica*, Hyacinth, *Fritillaria imperiales*, Yellow Narcissus, *Scillia sibirica*, *Galanthus nivalis* and *Crocus vernes*.

Muller-Thurgau[27] and Lendner[23] reported the disease from Switzerland. The latter observed that the newly formed bulbils may also be attacked.

Whetzel and Arthur[46] have given a good historical resumé and have studied the taxonomic relationships of the fungus. They say that the first indications of the disease are the bare spots in the tulip beds in the spring. Nearly all bulbs in the soil contaminated area are usually so injured that they fail to grow. When affected bulbs do send up leaves, their growth is greatly retarded, and they soon die and wither away. Initial infection evidently occurs in the fall and early winter shortly after the bulbs are put out into the beds, or early in the spring. When diseased bulbs are dug up, they are found to be more or less rotted, the infection being usually at the tip, or nose, of the bulb. The healthy white tissue is turned to a grayish or a reddish gray colour. The soil clings to the exterior of the rotted parts and embedded in the soil or in the rotted bulbs are sclerotia. Experiments showed that the pathogen depends upon its sclerotia to tide over from one season to the next. Mycelium, which is readily produced from the sclerotia, spreads through the soil and attacks the suscept. The pathogen appears to be a low temperature parasite.

Whetzel and Arthur remark that certain distinctive features in the morphology (especially in sclerotial structure and mycelial characters) of the pathogen show taxonomic relations to *Rhizoctonia solani* and *Corticium stevensii*. They regard these as sufficient to warrant its transfer from the genus *Sclerotium* to *Rhizoctonia*.

Brooks[5] gives an account of the disease of tulips and *Iris reticulata* caused by *Sclerotium* (*Rhizoctonia*) *tuliparum*. Tulips or other bulbous plants affected by this disease may be either completely destroyed below the soil level, or they put forth shoots which appear above the ground but are dwarfed and malformed and never flower. The sclerotia of the fungus cling to the neck of the bulb and the part of the shoot below soil level. The infection almost invariably proceeds from the soil by the formation of

strands of mycelium from sclerotia already therein. He found that Hyacinths, Daffodils, *Scillia sibirica*, *Fritillaria imperialis* and *Iris hispanica* are also attacked.

Dowson[15] reports that *Sclerotium tuliparum* sometimes attack tulips and *Iris reticulata* in England causing gray bulb-rot. He gives the usual symptoms of the disease. Infection is entirely due to contaminated soil and takes place in early winter. The parasite spreads but slowly from one place to another and is probably introduced into a new locality by a few small sclerotia embedded between the scales of otherwise perfectly sound bulbs.

Van Beyma Thoe Kingma[38] notes the frequent association of *Sclerotium tuliparum* with *Penicillium corymbiferum* on tulip bulbs, both of these being active parasites. Weber[45] gives the symptoms, etiology and control of sclerotial disease of tulips caused by *Sclerotium tuliparum*. Kawamura[19] states that tulip bulbs in Japan are liable to infection by *Sclerotium rolfsii* with which *Sclerotium tuliparum* is believed to be identical. Buddin[11,12] reports that *S. tuliparum*, besides attacking tulips, also occurs though generally less severely on Iris, *Scilla*, Crocus, *Ixia*, *Fritillaria*, *Colchicum*, Hyacinth and Narcissus. Observations showed that bulbs planted with one-half to two-thirds of their surface protruding mostly remained healthy even in badly diseased soil. Steaming of soil completely eliminated the disease. In the control of the disease when a powder containing chloronitro-benzol was mixed with the surface soil 90 per cent. control was obtained whereas sprinkling the soil after planting the bulbs was unsatisfactory.

For the first time in 1938 *Sclerotium tuliparum* was recorded in England and Wales on Crocus.[49] Osterwalder and Camenzind[33] tested 0·5 per cent. formalin solution against *S. tuliparum* on tulips with satisfactory results.

Control measures, which rely chiefly on chemical disinfection of bulbs or soil, have been described by Caballero,[13] Wakker,[41] Ritzema Bos,[35] Klebahn,[22] Whetzel and Arthur,[46] Dowson,[15] Van Slogteren,[44] Buddin[11,12] and Osterwalder and Camenzind.[33]

White rot of *Allium* is a disease of widespread occurrence and was first recorded by Berkeley[4] in 1841 in Great Britain who named it *Sclerotium cepivorum* Berk. Voglino[39] recorded severe attack of leeks in Italy by *S. cepivorum* but on the basis of his cultural study he renamed the fungus *Sphacelia allii*. Cotton and Owen[14] reported that the white rot disease of onion bulbs caused considerable damage to onion crops in Great Britain. They found that shallots were markedly resistant and leeks did not appear to suffer. Caballero[13] reports considerable damage in garlic fields in Spain

and regards *Sclerotium cepivorum* as the most destructive of the garlic parasites.

Walker in a series of papers[42,43,44] described the white-rot of *Allium* caused by *S. cepivorum* in Europe and America and found that the disease occurred on onion, Welsh onion, leek, garlic and shallot. Leeks only suffered from the disease during cooler months. The fungus attacked the plants at any time during the growing period, provided external conditions were favourable. He observed that the disease thrives best at moderately cool temperatures and with moderate soil moisture. He found that within the temperature range favourable to growth of the plant, the fungus became less destructive as the rapidity of host-growth increased.

Dowson[15] gives a brief description of *Sclerotium cepivorum* and its host. He also observed that warm and damp weather favours the disease which is spread by the planting of diseased seedlings or sets and is increased by repeatedly planting onions in the same ground. Nattrass[28,29] reports the occurrence of the disease from Egypt and Cyprus. He describes its symptoms and says that overwintering is due to sclerotia in the soil and that the fungus did not grow above 30° C. He further suggests that sets should only be planted from disease-free areas and cultivation of the different species of *Allium* should be discontinued for 8 to 10 years.

Du Plessis[16, 17] gave a popular account of white mould on onion caused by *S. cepivorum* in South Africa and reports that disinfection of soil by formalin, heat or mercuric chloride are impracticable on a large scale though in laboratory test the sclerotia, which persist in soil and onion refuse for four or more years, succumbed to these treatments. He found that losses may increase from 20 to 30 per cent. when pink rot and bulb rot are accompanied by white mould caused by *S. cepivorum*.

Onion varieties showing marked resistance to white rot have been developed at Manchester University.[48] Matzulevitch[25] gives very brief description of the disease occurring in Russia. From one locality in Czechoslovakia an epidemic outbreak of *Sclerotium cepivorum* on garlic is reported.[47] Marchionatto[24] reports that *S. cepivorum* has been recognised since 1913 on onions and garlic in Argentina.

Bremer[8,9] reports the presence of the disease in Germany and also recommends a well-regulated rotation in which onions are excluded from infested fields for at least 8 to 10 years. He has also given a popular note on the rots of stored onions in Germany by *S. cepivorum* and other organisms. Bremer and Nicolaisen[10] have given symptoms, etiology and control of the disease. In New South Wales (Anon[1]) white rot has been recorded once on garlic and thrice on onions.

Ogilvie and Hickman[30] found the disease widely distributed in Bristol province, mainly on white Lisbon spring onion. A soil application of a proprietary organic mercury compound in dust form, containing hydroxymercurichlorophenol with 20 per cent. organically combined mercury, before sowing, gave 56·8 and 17·9 per cent. infection at two localities respectively against average of 86·7 and 90·4 per cent. for the corresponding untreated control plots. Ogilvie, Croxall and Hickman[31] report that early autumn sowing of onions were more severely affected by white rot than were late sowings. Ogilvie and Walton[32] note that Up-to-Date, Rousham Park Hero, Improved Reading and White Spanish onions are moderately resistant, leeks being only occasionally attacked.

Brandão[7] has given the symptoms and control of white rot affecting garlic in Brazil. The disease has also been recorded in Argentina by Hauman-Merck,[18] in United States by Valleau,[37] in Holland by Van Poeteren[34] and in various parts of Australia.[1]

Asthana[2] states that high potash manuring in England showed some decrease in the attack of *S. cepivorum* on onions but there was indication from plot experiments that liming reduced considerably more the incidence of the disease on onion seedlings. Moore[26] reports the fungus to be seed-borne and is usually transmitted by infected seedlings. Booer[3] found that application of 4 per cent. mercurous chloride (calomel) dust to the seed drill at sowing time gave better results than seed treatment. One lb. of dust per 25 yd. of seed drill gave good disease control in bulb onions, and one lb. per 50 yd. may suffice for salad onions.

Material and Method

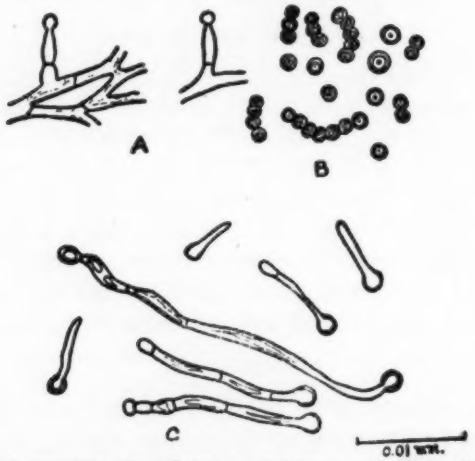
All through the experimental work pure cultures of *Sclerotium cepivorum* and *Sclerotium tuliparum* were used. Isolations were made from diseased onion and tulip bulbs and the purity of all the cultures was assured by the single hyphal tip method of Brown.[6] All the cultures were maintained on potato-dextrose agar. Throughout the laboratory and field experiments "White Spring Lisbon" onion seeds, English onion bulbs and "Prince of Austria" tulips were used. The use of other varieties of onion and tulip will be mentioned at the appropriate places. Field and pot culture experiments were carried out at Slough and at Chelsæ Physic Garden respectively.

Morphology

Mycelial and sclerotial growths of *Sclerotium cepivorum* and *Sclerotium tuliparum* were made on potato-dextrose agar plates. *S. cepivorum* forms a fluffy white mycelial growth, which is rather coarse with large cells. The

branches often anastomose and hold the hyphæ together in sheets or strands. The growth of the fungus is quite vigorous. After ten days of incubation at 20° C., sclerotia first appear as small white tufts of loosely intertwined branches. They become circular in form and within one to two days change into dull green from white. The outer layers darken and after a further two days the sclerotia appear as hard black bodies of 0·6 to 0·8 mm. diameter. Within two weeks of inoculation they are formed in large numbers over the whole surface of the plate (Plate III, Fig. 1). In section the sclerotia show a black cortex and inside a medulla of elongated closely packed hyphæ, in this respect resembling the sclerotium of *Sclerotinia sclerotiorum*.

The only spores produced by *S. cepivorum* were microconidia. Voglino[39] in the cultural study of the organism has described the production of sporodochia of hyaline conidiophores upon which were borne spherical, hyaline and catenulate conidia. Presumably the conidia of Voglino were the microconidia. It was observed that the microconidia were produced on certain media only, *e.g.*, six week old plates of Brown's Starch agar and three to four week plates of tulip agar. These microconidia are formed on small conidiophores, 6–10 μ long, and are spherical, 2·5 μ–3·4 μ in diameter, with two walls. Very often they occur in chains, 2–8 sticking together, which arise by successive constrictions of the conidiophores. Attempts to germinate these microconidia almost uniformly failed. In a very few cases, about 14 spores altogether, short septate germ tubes appeared but these soon cut off a microconidium at the tip and ceased to grow. The germination was observed only on 20 per cent. Tulip juice (Text-Fig. 1).



TEXT-FIG. 1. Microconidia of *Scleeotium cepivorum*. A—Formation of microconidia. B—Microconidia. C—Germinating microconidia.

The hyphæ of *Sclerotium tuliparum* are long, slender and septate, the individual cells tending to be barrel-shaped when young. The mycelium grows rhizomorphically at a fairly uniform rate. It is mostly white, at first appressed and somewhat silky, later becoming more distinctly aerial towards the periphery. With age the colour of the mycelium gradually changes from white to clay. The medium soon becomes discoloured, taking on a distinctly reddish-brown tinge which deepens with age. The sclerotia first appear after 10 days as irregular, white, cottony masses on the surface of the culture, in a broad ring near the periphery of the colony (Plate III, Fig. 2). They soon turn to a pale yellow, deepening to reddish-brown, and becoming almost black when dry. They are generally globose to oblong bodies, 3–4 mm. in diameter but they vary a lot in their size and form with different media. In many cases several scelerotia are agglomerated into a large, irregular mass and in others the size varies from 1·5 to 8 mm. in diameter. In contrast to that of *S. cepivorum*, the surface of the sclerotium is dull, rough and irregular. In cross-section the medulla is seen compact and definite in form, having globose cells.

Growth

Nutrient Media.—Both the fungi under study were grown on 28 different natural and synthetic media. Petri-dishes of an equal depth were poured, inoculated at the centre with *S. cepivorum* and *S. tuliparum*, and incubated at 20° C. for two weeks. The results of the comparative study of the mycelial and sclerotial growths are given in Table I.

Table I shows that both fungi grow more or less freely on a large variety of media and that in general the richer the medium the greater the mycelial and sclerotial development. *S. cepivorum* growth is favoured by an acid medium whereas *S. tuliparum* prefers a neutral or alkaline one. A representative set of growth form is illustrated in Plate III, Figs. 1 to 4 and Plate IV, Figs. 5–6.

Throughout the series the mycelium of *S. cepivorum* is white and woolly while that of *S. tuliparum* is clay coloured and appressed. Characteristic features of *S. cepivorum* and *S. tuliparum* respectively are the peculiar sweet musky odour in all the cultures and the discolouring of the medium which takes a reddish-brown tinge with age.

To a certain extent the number of sclerotia produced on the same medium varies with the depth of pouring. Table II gives the number of sclerotia of *S. tuliparum* per plate as counted by the naked eye while in the case of *S. cepivorum* the numbers refer to a standard microscopic field as the sclerotia are minute.

TABLE I

Comparative study of the mycelial and sclerotial growths on a variety of nutrient media

Media	*S. cepivorum*		*S. tuliparum*	
	Mycelial Growth	Sclerotial Growth	Mycelial Growth	Sclerotial Growth
Potato extract ..	++	++	++	++
Potato mush ..	+++	+++	+++	++
Turnip agar (20%) ..	+++	+++	+++	+++
Tulip agar (20%) ..	++++	++++	++++	+++++
Pea agar (20%) ..	+	+	+++	+++++
Oat meal agar ..	+	+	++	+
Malt agar ..	+	+	+	+
Prune agar ..	++	++	+	+
Lettuce agar (20%) ..	++	++	++	++
Leek agar (20%) ..	++	+++	+	+
Onion agar (25%) ..	++	+++	++	++
do (20%) ..	+++	++++	++	++
do (15%) ..	++	+++	++	+
do (10%) ..	++	++	+	+
do (7·5%) ..	++	++	+	+
do (5%) ..	+	+	+	nil
do (2·5%) ..	+	+	+	nil
Brown's Starch agar ..	+++	+++	++	++
Asparagin glucose	+	+	++	+
Acid Asparagin glucose ..	+++	++	+	+
Glucose peptone ..	++	++	+	nil
Glucose nitrate ..	++	++	+	+
Glucose NH_4NO_3	++	++	++	+
Glucose NH_4 tartrate ..	++	+	+	nil
Cane-sugar nitrate agar ..	++	+	+	+
Coon's agar ..	+	+	+	+
Chohn's nutrient agar ..	++	+	+	+
Richard's agar ..	++++	+++	++++	+++

[+ = Scanty or a few; ++ = moderate; +++ = good; ++++ = abundant sclerotia and thick mycelial growth; +++++ = luxuriant mycelial growth, large and abundant sclerotia.]

TABLE II

Amount of medium per standard plate	Number of sclerotia of *S. tuliparum* after 2 weeks		Number of sclerotia of *S. cepivorum* per microscopic field after 2 weeks	
	Mature	Immature	Mature	Immature
20 c.c. ..	136	19	2·5	1·2
40 c.c. ..	300	47	5·0	2·0

Here it will be seen that with the depth of the medium (Brown's Starch agar) the number of sclerotia is increased in both the fungi while increase

in size was only observed in case of *S. tuliparum*, no such effect being produced on the sclerotia of *S. cepivorum*.

Similarly the depth of plating influences the rate of linear growth. The figures in Table III give the increase in growth on Brown's Starch agar from the 4th to the 8th day. The effect is marked in the case of *S. tuliparum*, but negligible in the other.

TABLE III

Amount of medium per standard plate	*S. tuliparum*	*S. cepivorum*
10 c.c. ..	1·6 cm.	3·6 cm.
20 c.c. ..	2·3 cm.	3·8 cm.
40 c.c. ..	3·4 cm.	4·2 cm.

By referring to Table I it will be observed that media prepared from onion extracts were more favourable to the growth of *S. cepivorum* than to that of *S. tuliparum*. This point was investigated in greater detail.

The juice of onions and of tulips was squeezed out under a hand press, filtered through muslin and centrifuged to remove the coarse particles. Various dilutions of those extracts were then made and drops placed on cover slips in Ward-cells or on slides in moist petri-dishes. These petri-dishes contained a layer of agar to which 0·4 per cent. mercuric chloride was added and the slides were laid on this. By this method a moist atmosphere was maintained and the development of contaminating fungi and bacteria was reduced to a minimum. Each nutrient drop was inoculated with a hyphal tip of *S. cepivorum* or *S. tuliparum*. Table IV records the state of growth after 24 hours at 20° C.

TABLE IV

Mycelial growth in concentrations of crude onion juice

Fungus	Percentage of concentration of crude onion juice					
	10	20	40	60	80	100
S. cepivorum ..	++	+++	++++	+++	++	+
S. tuliparum ..	nil	nil	nil	nil	nil	nil

[+ = scanty ; + + = moderate ; + + + = good and thick ; + + + + = luxuriant.]

The main point brought out is that *S. tuliparum* does not grow on any of the dilutions of crude onion extract (pH 6·0). The same was true after

60 hours. It is noteworthy that *S. cepivorum* is also considerably affected in its growth at the higher concentrations of the extract.

When however the onion extract was steamed for half an hour before use, the differential effect shown in Table IV was much lessened.

TABLE V

Mycelial growth in concentrations of steamed onion juice after 24 hours

Fungus	Percentage of concentration of steamed onion juice					
	10	20	40	60	80	100
S. cepivorum ..	++	+++	+++	+++	++++	++++
S. tuliparum ..	+++	++	++	++	+	+

(Notation as in Table IV.)

S. tuliparum grew quite well on this medium, especially at the lower concentrations, and the retarding effect of high concentration on the growth of *S. cepivorum* also disappeared.

That the effect of boiling was the dissipation of an inhibitory volatile substance was shown by studying the growth of the two fungi in turnip extract (20 per cent.) in the presence of crude unboiled or boiled onion extracts. These experiments were carried out in hanging drops, the turnip extract with mycelial tip being on the cover slip, and the bottom of the cell containing the onion extract. The comparative results are shown in Table VI.

TABLE VI

Mycelial growth after 24 hours on turnip extract in the presence of crude and boiled onion extracts

Fungus	Crude onion extract	Boiled onion extract
S. cepivorum ..	+	+++
S. tuliparum ..	nil	++

(Notation as in Table IV.)

The corresponding results with tulip juice, (*a*) unboiled (pH 5·8), (*b*) boiled (pH 6·2) are shown in Table VII (Notations as in Table IV). On boiling the juice a precipitate was formed which was removed and the growth of the two fungi was observed in the clear filtrate,

TABLE VII

Mycelial growth in different concentrations of tulip juice after 48 hours

Fungus	Percentage of concentration of tulip juice					
	10%	20%	40%	60%	80%	100%
			Unboiled juice			
S. cepivorum ..	++	+	nil	nil	nil	nil
S. tuliparum ..	++++	+++	+++	++	++	+
			Boiled juice			
S. cepivorum ..	+	+	nil	nil	nil	nil
S. tuliparum ..	++	+++	+++	++	+	+

In contrast to the results with onion juice, boiling has no obvious effect in making tulip juice more suitable for the growth of *S. cepivorum*.

The conclusions arising from Tables IV–VII are that crude onion juice is highly inhibitory to the growth of *S. tuliparum* but that this inhibition is removed by boiling. On the other hand, tulip juice is relatively unfavourable for the growth of *S. cepivorum* and this effect is not removed by boiling.

The fact that *S. cepivorum* and *S. tuliparum* prefer the extracts of their particular host-plants is also shown, though not so markedly as in Tables IV–VII, by studying their growth rates on agar media compounded with these extracts. Comparative data after 48 hours are given in Table VIII.

TABLE VIII

Growth rates in cm. on different concentrations of onion and tulip agar media after 48 hours

Percentage of concentration of the medium	*S. tuliparum*		*S. cepivorum*	
	Onion	Tulip	Onion	Tulip
2·5	1·0	3·0	2·9	2·8
5·0	1·4	2·9	3·4	3·2
10·0	1·6	2·4	4·0	3·0
15·0	1·7	2·7	4·7	2·7
20·0	2·1	2·4	5·1	3·0
25·0	1·8	2·2	4·5	3·0
30·0	1·3	1·8	3·8	3·9
35·0	1·1	2·0	3·2	2·5

Here it is seen that each fungus grows somewhat more rapidly on an agar medium prepared from the juice of its own host plant.

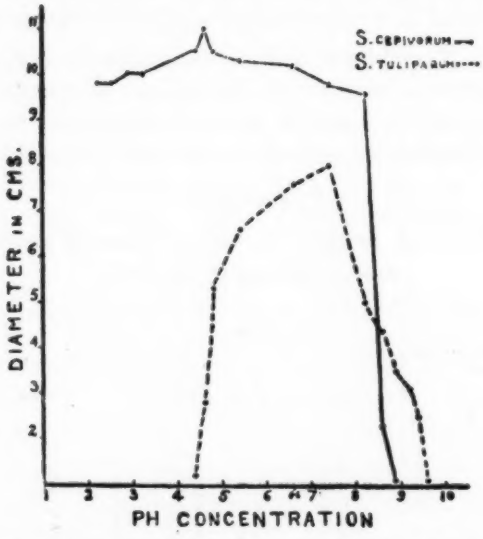
Table IX, which gives the growth of *S. cepivorum* and *S. tuliparum* after 10 days at 20° C. on 20 per cent. extracts of the juice of Spanish and English onions, Spring onion leaves and Spring onion bulbs further illustrates the relatively slow growth of *S. tuliparum* on onion media.

TABLE IX

Growth of the colonies in cm. on different onion media

Fungus	Spanish onion	English onion	Spring onion leaves	Spring onion bulbs
S. cepivorum ..	8·0	7·8	6·5	5·4
S. tuliparum ..	2·8	2·7	2·3	1·9

H-ion concentration.—The two fungi were grown on plates of Brown's Starch agar (pH 6·6). The medium was adjusted to different pH values by adding malic acid or sodium bicarbonate, which were separately autoclaved and added just before pouring. Inoculations of the plates were made in the centre by a single sclerotium in the case of *S. tuliparum* and three in case

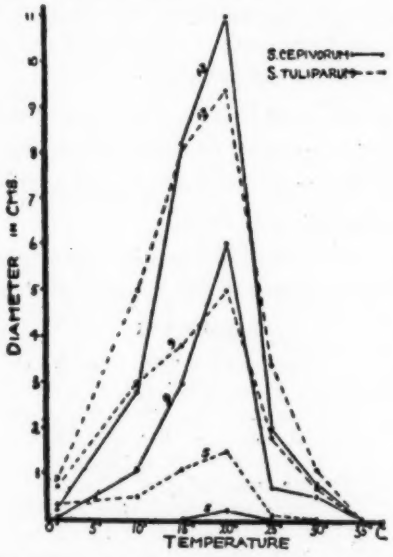


TEXT-FIG. 2. Illustrating growth of *S. cepivorum* and *S. tuliparum* at different H-ion concentrations.

of *S. cepivorum*. All the plates were incubated at 20° C. for 12 days when the colony growths were measured. The results are shown in Text-Fig. 2, in which each reading is an average of ten diameters from five different plates,

It will be seen from the figure that the growth of *S. cepivorum* is fairly constant over a wide range (2·2–8·2) of H-ion concentration. A higher pH than 8·2 causes the growth rate to fall considerably. On the other hand the curve for *S. tuliparum* shows a well-defined optimum near the neutral point. The range of *S. tuliparum* in the alkali side is greater than that of *S. cepivorum*, and conversely for the acid side.

Temperature.—The temperature response of the two fungi on potato-dextrose agar over the range 1°–35° C. is shown in Text-Fig. 3. Each point in the curves represents the average of ten measurements taken from five-fold series of plates on the 5th, 9th and 13th day.



TEXT-FIG. 3. Illustrating effect of Temperature on Growth of *S. cepivorum* and *S. tuliparum.*

The optimum temperature for both fungi is near 20° C., the minimum somewhere near zero and the maximum between 30° and 35° C. At the optimum temperature, *S. cepivorum*, though slower in beginning growth, rapidly out distances the other. At temperatures removed from the optimum, both above and below, *S. tuliparum* is the faster grower.

Light.—The effect of light factor was tested on cultures growing on 20 per cent. onion agar, Brown's Starch agar, 20 per cent. turnip agar and Richard's agar but no significant difference was observed either in growth rate or in the general appearance of the cultures.

Summary

1. Brief accounts of the literature dealing with the fungi *Sclerotium cepivorum* Berk. and *Sclerotium tuliparum* Klebahn are given.

2. Morphology of the two fungi has been described. The only spores produced by *S. cepivorum* are microconidia on certain media only. Attempts to germinate these microconidia almost uniformly failed as only 14 spores altogether germinated on 20 per cent. tulip juice.

3. A comparative cultural study of *S. cepivorum* and *S. tuliparum* showed that while both grew well on a great variety of media, nevertheless there was in the case of each a certain amount of specific reaction to the juice of its own host plant. In particular, crude onion juice is markedly inhibitory to the growth of *S. tuliparum*. Boiling of the juice largely removes this effect. *S. cepivorum* does not grow well in tulip juice, boiled or unboiled.

4. *Sclerotium cepivorum* is favoured by an acid reaction of the culture medium while *Sclerotium tuliparum* by a neutral or slightly alkaline reaction.

5. The temperature range of growth for both fungi is approximately 1°–35° C., with an optimum near 20° C.

6. There is no significant difference by light factor either in growth rate or in the general appearance of the cultures of the two fungi.

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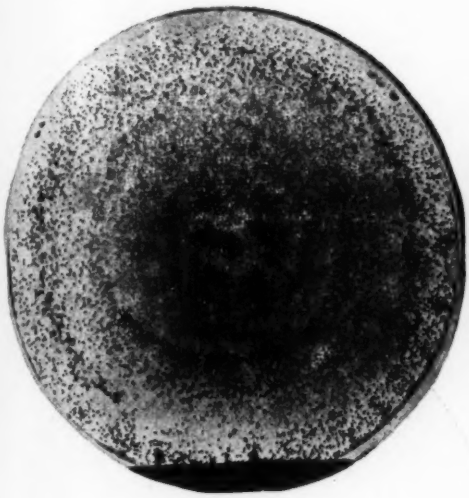

FIG. 1

FIG. 2

FIG. 3

FIG. 4

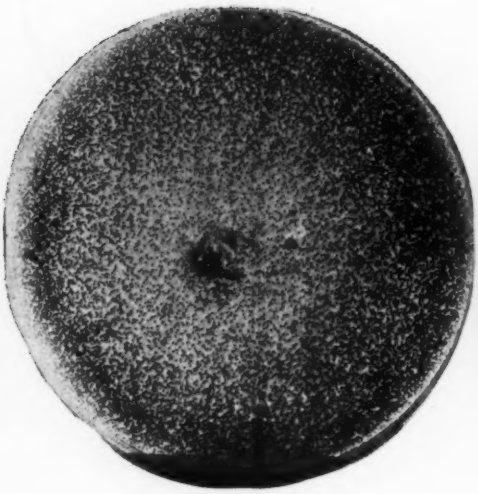

FIG. 5

FIG. 6

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Explanation of Plates

Figs. 1, 3 and 5—Plate cultures of *Sclerotium cepivorum* on potato-dextrose agar, tulip agar and onion agar respectively.

Figs. 2, 4 and 6—Plate cultures of *Sclerotium tuliparum* on potato-dextrose agar, tulip agar and onion agar respectively.

STUDIES ON SCLEROTIUM-FORMING FUNGI

I. *Sclerotium cepivorum* Berk and *S. tuliparum* Klebahn

Part 2. Symptoms, Mode of Infection and Host Range

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Introduction

Bulb-rot of tulips and white-rot of onions were first reported by Wakker[22] in Holland and Berkeley[3] in Great Britain respectively. Bulb-rot, caused by *Sclerotium tuliparum* Klebahn, was subsequently recorded in Germany by Klebahn,[13] in Switzerland by Muller-Thurgau[18] and Lendner,[14] in United States by Whetzel and Arthur[24] and Buddin,[7] in England by Brooks[6] and Dowson,[10] in Holland by Van Beyma Theo Kingma[20] and in Japan by Kawamure.[12] Similarly white-rot, caused by *Sclerotium cepivorum* Berk, was later reported in Italy by Voglino[21], in Spain by Caballero,[8] in Europe and America by Walker,[23] in Egypt and Cyprus by Nattrass,[19] in South Africa by Du Plessis,[11] in Russia by Matzulevitch,[16] in Argentina by Marchionatto,[15] in Germany by Bremer,[5] in Brazil by Brandão[4] and in various parts of Australia (Anon,[1–2] 1938, 1943).

Symptoms and Mode of Infection

The earliest signs of attack of *S. cepivorum* on spring-sown seedling onions usually become noticeable about the end of May or beginning of June. The older leaves first turn yellow, starting from the tips downwards, and then fall over. The inner leaves collapse later on. Affected plants can readily be pulled from the soil, because the stem base is more or less completely rotted. Around the base of affected bulbs a white, fluffy mycelial growth is frequently seen. Later on sclerotia are formed on or inside the scales as the mycelium penetrates into the interior and gradually destroys the base and the scales so that the bulb becomes rotten and worthless (Plate V, Figs. 1–3). The fungus causes a semi-water decay of the scales. The sclerotia formed are minute, black and hard. Attack is not limited to young seedlings, but one frequently finds large bulbs in the autumn showing early stages of attack.

Hyphæ of the fungus can readily be demonstrated ramifying through all the disintegrated tissue, roots, stem and leaf bases.

When onion seed is sown in ground artificially contaminated by the fungus, *e.g.*, by placing the seed on pieces of the fungal mycelium in the ground, more drastic attack may be shown. In such a case a large percentage of the seedlings may fail to come above ground.

The fungus may be seed-borne, as reported by Moore,[17] but is usually transmitted by infected seedlings, and distributed locally by cultivation. Cotton and Owen[9] suggest that in all cases the roots are attacked before the bulbs, but in the writer's opinion this is not the case. Field observation shows from time to time bulbs with the appearance illustrated in Plate V, Fig. 4. In such a case the base of the bulb is entirely rotted though a large percentage of the roots are still not invaded. Unless special percautions are taken such a bulb when pulled will tear apart at the stem base, part of the latter being still anchored to the ground by the healthy roots.

Inoculation experiments carried out in the laboratory support the view that the stem base is the part of the bulb most susceptible to invasion. Healthy bulbs, with the leaves cut off were suspended, after careful washing and surface sterilisation, in moist glass jars and inoculated either—(*a*) on the uninjured surface of scale leaves; (*b*) on the injured surface of scale leaves; (*c*) on the uninjured stem base at the point of emergence of roots; (*d*) on the uninjured root surfaces. Ten bulbs were tested in each lot. The results are indicated in Table I.

TABLE I

Rot on the uninjured surface of scale leaves	Rot on the injured surface of scale leaves	Rot on the uninjured stem base at the pint of emergence of roots	Rot on the uninjured root surface
Nil	+ +	+ +	+

While the fungus appears to be able to penetrate the surface of uninjured roots, it does so relatively slowly and uncertainly whereas it freely enters at the stem base. The port of entry has been found to be the natural wound caused by the emerging root. It is to be noted that no attack takes place through the surface of the intact outer scales.

The symptomatology of "gray-bulb rot" of tulips due to *S. tuliparum* is somewhat different from that of the "white-rot" of onions by *S. cepivorum*. In the field the disease is first indicated in the spring by the failure of bulbs in certain patches to appear above ground. Bulbs which are less severely attacked may send up some distorted leaves but as a rule no flower is formed. When such plants are dug up, one finds that the rot is at the

base of the leaves, *i.e.*, at the nose of the bulb and that the leaves are only attached to the bulb by a thin brown connection of rotted tissues. In the cases where bulbs fail to come up, one is struck by the fact that soil clings to the exterior of the rotted part. The roots of a badly infected bulb may be perfectly sound. The rot is of a dry type and the healthy white tissue turns to a reddish gray colour and becomes brittle. Sometimes brownish-black sclerotia are formed inside the bulb scales but more usually they occur in the soil adhering to the bulbs. In advanced stages of infection the mycelium forms a felty layer between the scales of the bulbs.

The fact that tulips when planted in infected grounds at the normal depth (about 5 inches) are attacked at different stages probably indicates that the base of the shoot is infectible over a considerable period of time. If however the bulbs are planted very shallow, so that their noses are just below ground level, the base of the shoot passes through the susceptible stage rather quickly. This is illustrated in Table II which gives the results of an experiment with potted plants.

TABLE II

(20 bulbs were planted and inoculated in each case)

Description of inoculation	No. of plants growing above soil level	No. of plants flowering
1. Control (uninoculated) ..	20	19
2. Bulbs inoculated at nose when shoots are 1–1½ inches long ..	5	Nil
3. Bulbs inoculated at nose when shoots are 2½–3 inches long	17	15

Under such conditions the base of the shoot presumably becomes harder with thicker cuticle than when the bulb is deeply planted.

Attempts in the laboratory to infect portions of the flowering stem above ground or the leaf-blades, wounded or unwounded, were uniformly negative.

Pot experiments indicated that the roots are not directly attacked by the fungus. Thus in one case 20 bulbs were planted over the fungal mycelium, while 20 others had the mycelium placed on the nose. Of the latter 5 only grew and 2 produced flowers, of the former 16 flowered. In all cases it was the shoot bases and not the roots which were attacked.

The experiments on influence of soil moisture and temperature on infection were mostly confined to the attack of *S. cepivorum* on white spring onion (Lisbon variety).

The soil, a mixture of medium loam, sand and leaf-mould, was autoclaved and dried down. The water-holding capacity of such a soil was found to be 46 per cent. of the dry weight. Six batches of this soil were then adjusted to moisture contents of 100, 80, 60, 40, 20 and 10 per cent. of water-holding capacity. These were placed in varnished earthen pots 6″ in diameter and with a depth of soil of about two inches. The pots were placed under bell-jars and sufficient water added daily to each to keep up a constant weight. The results of two sets of such experiments are given in Table III when the seedlings had grown for eight weeks.

TABLE III

Percentage of soil moisture	Experiment I		Experiment II	
	No. of plants growing in uninoculated soil	No. of plants growing in soil inoculated with *S. cepivorum*	No. of plants growing in uninoculated soil	No. of plants growing in soil inoculated with *S. cepivorum*
100	65	40	70	36
80	67	25	66	30
60	50	12	47	15
40	36	2	32	5
20	12	10	18	12
10	Nil	Nil	Nil	Nil

These results indicate that *S. cepivorum* is able to attack onion seedlings over the whole range at which ready germination takes place. There is some suggestion also that the greatest development of the disease is near about 40 to 60 per cent. soil moisture, higher or lower percentages reducing the disease.

The effect after six weeks of varied soil temperature is shown in Table IV. The experimental pots were placed in a range of green-houses. The temperatures were not under very strict control.

TABLE IV

(Number of seeds used was 90 in each case)

Temperature in Centigrade	Number of healthy plants growing		
	Control	*S. tuliparum*	*S. cepivorum*
25°–30°	21	18	15
13°–18°	74	72	24
8°–13°	65	66	32
2°—8°	55	53	46

The figures indicate an optimum of attack somewhere in the neighbourhood of 13°–18° C. At the higher temperatures, germination was very poor even in the controls, but there was relatively little attack. At the lower temperatures germination was good, and there was little attack.

The good agreement shown between the series which was uninoculated and the one which was inoculated with *S. tuliparum* indicates, as before, that this fungus causes no attack.

Host Range

An experiment was set up in a green-house in which 20 tulip bulbs were planted early in December in pots, five per pot, (*a*) without addition of fungus, (*b*) with mycelium of *S. cepivorum* at top and bottom of bulbs, and (*c*) with mycelium of *S. tuliparum* placed as in (*b*). Observation five months later gave the results shown in Table V.

Table V

(20 tulip bulbs were planted in each case)

Fungus	No. of plants grown	No. of plants flowering
Soil uninoculated ..	20	18
S. cepivorum ..	20	18
S. tuliparum ..	6	5

There was thus no evidence that *S. cepivorum* had produced any effect.

This experiment was repeated on a larger scale in the following year in the open ground. 12′ × 12′ area was divided into 12 rows, and in each row 20 Prince of Austria tulip bulbs were planted at a distance of six inches apart. The first row was uninoculated, the second inoculated by *S. cepivorum* at the top and at the base of the bulbs and the third similarly by *S. tuliparum*. This scheme was replicated four times so that altogether 80 bulbs were subjected to each treatment. All the bulbs were planted and inoculated in November. The number of plants which had come above ground were counted in March and the number of plants flowering were recorded after another two months. The data are given in Table VI.

It is thus clear that under conditions which were sufficiently favourable to enable *S. tuliparum* to produce nearly 100 per cent. infection, *S. cepivorum* had no ascertainable effect whatsoever. None of the bulbs inoculated with *S. cepivorum* showed any trace of the latter fungus, either on the scales or on the roots, at the time of lifting.

TABLE VI

(80 bulbs were planted in each case)

Fungus	No. of plants appearing above ground	No. of plants flowering
Soil uninoculated ..	75	73
S. cepivorum ..	78	78
S. tuliparum ..	4	1

From laboratory experiments, it did not appear that *S. cepivorum* could attack tulip tissue even when the epidermis was removed. Some growth of the fungus took place but there was no obvious effect on the bulb tissue.

The behaviour of the two fungi towards onion plants was tested in a series of pot experiments, the results of which are set out in Tables VII and VIII. 80 seeds were sown in drills, with or without inoculation, in each case in the unautoclaved soil series while 108 in the autoclaved one. More extended tests which gave substantially the same conclusions were carried out on a field plot scale and the results are given in Table IX.

TABLE VII

(Ten autumn-sown sets were planted in each case)

Treatment	Number of plants grown	Number of plants healthy
Uninoculated soil ..	10	10
Inoculated by *S. cepivorum* ..	6	4
Inoculated by *S. tuliparum* ..	9	9

TABLE VIII

Percentage of growth

Treatment	Unautoclaved soil	Autoclaved soil
Uninoculated soil ..	72·0	82
Inoculated by *S. cepivorum* ..	42·7	30
Inoculated by *S. tuliparum* ..	67·0	76

On account of a certain amount of variation in the percentage germination of the seeds, the results are not so clear cut as in the converse case of tulip bulbs described above. The two tables however show definitely that *S. tuliparum* produces no attack of onion sets or seedlings under conditions

where *S. cepivorum* caused the loss of approximately 50 per cent. of the plants.

A comparison of the results of the Table VIII suggests that the capacity of *S. cepivorum* to attack is somewhat greater in autoclaved than in ordinary soil.

TABLE IX

(400 onion seeds were used in each case)

Treatment	Percentage of healthy plants
Control (uninoculated) ..	43·0
S. tuliparum ..	48·0
S. cepivorum ..	25·0

It appears therefore from these experiments that neither *S. cepivorum* nor *S. tuliparum* is able to attack the host of the other.

A series of inoculations was carried out with *S. tuliparum* and *S. cepivorum* on a miscellaneous assortment of plants possessing bulbs, corms, etc. Inoculations were made or wounded or unwounded materials either in moist chambers or in the soil of pots. The following is a summary of the results obtained.

(*a*) *S. tuliparum* caused 80–100 per cent. infection of Single Early tulip (Artus), Single Tulip (Prince of Austria), *Scilla sibirica*, Hyacinth (Crimson), *Chionodoxa luciliæ*, *Iris hispanica* (King of Whites).

(*b*) *S. tuliparum* caused infection in 40–60 per cent. of Gladiolus (peach Blossom), Narcissus (*Poeticus ornatus*), Daffodil (Princeps), Crocus (Light Blue), Snowdrop (Single). Rhizomes of winter Aconite was less frequently attacked, only 20 per cent.

(*c*) *S. tuliparum* produced no attack on English and Spanish mature onion bulbs, Spanish and English grown autumn sown sets, seedlings of white Lisbon and Red onion, Shallots, Leek (Musselburgh).

(*b*) *S. cepivorum* was not seen under any conditions to attack any of the plants listed under (*a*) or (*b*), whereas it vigorously attacked most of the onion types given under (*c*). Red onions, leeks and shallots were attacked to an extent of 20–25 per cent. only, *i.e.*, less than the other onion types.

It was noticed with both fungi that moist atmospheric conditions and autoclaved soil increased the pathogenicity on almost all the hosts.

The symptoms and mode of infection of both fungi on the above hosts are almost the same as described above for the natural hosts, *i.e.*, *S. cepivorum*

attacks the base of the bulbs and *S. tuliparum* the top and young growing hoots.

Summary

1. Symptoms on the natural hosts and the modes of infection of *S. cepivorum* and *S. tuliparum* are described. While *S. cepivorum* appears to be able to penetrate the surface of uninjured roots, it does so relatively slowly and uncertainly whereas it freely enters at the stem base. The port of entry has been found the natural wound caused by the emerging root. In case of *S. tuliparum* it was the shoot bases and not the roots which were attacked.

2. *S. cepivorum* is able to attack onion seedlings over the whole range of soil moisture at which ready germination takes place—the greatest development of the disease being near about 40 to 60 per cent. soil moisture. As regards temperature effect, the optimum attack is somewhere in the neighbourhood of 13° to 18° C.

3. Under conditions which were sufficiently favourable to enable *S. tuliparum* to produce nearly 100 per cent. infection on tulips, *S. cepivorum* had no ascertainable effect whatsoever.

4. Neither *S. cepivorum* nor *S. tuliparum* is able to attack the host of the other.

5. *S. tuliparum* caused 80–100 per cent. infection of Tulips, *Scilla sibirica*, Hyacinth, *Chionodoxa luciliæ*, *Iris hispanica*; 40–60 per cent. of Gladiolus, Narcissus, Daffodil, Crocus, Snowdrop; 20 per cent. of rhizomes of winter Aconite; and produced no attack on onions, shallots and leek. *S. cepivorum* on the other hand attacked vigorously most of the onion types, leeks, shallots and red-onions only up to an extent of 20–25 per cent., while it could not attack the hosts of *S. tuliparum*.

6. It was noticed with both fungi that moist atmospheric conditions and autoclaved soil increased the pathogenicity on almost all the hosts.

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Explanation of Plate

Fig. 1. Two healthy spring onion bulbs with roots.

Fig. 2. Two diseased spring onion plants. The black covering all round the base in the mycelial mat and sclerotia of the pathogen.

Fig. 3. An advanced case of attack of *S. cepivorum*. The photo shows the whole plant of a spring onion, the leaves have all dried and fallen off.

Fig. 4. An early stage of infection by *S. cepivorum* on onion bulb. Though the base of the bulb is rotted yet only a few roots have gone. A high percentage of healthy roots is shown here.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

STUDIES ON SCLEROTIUM-FORMING FUNGI

I. *Sclerotium cepivorum* Berk and *Sclerotium tuliparum* Klebahn

Part 3. Pectinase Activity and Preparation

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There are certain factors which condition invasion of particular hosts by some fungi and not by others. The chief relevant references are given below.

Walker, Lindegren and Bachmann[6] report the presence of toxic substances in the juice extracted from succulent onion scales. They remark that these toxins are of two general types, one which is neither removed nor broken down readily by heat and one which is volatile and passes off from the extracted juice at room temperature within a few hours. There is a gradual decline during storage of onion bulbs in the amount of volatile toxin, a decline which is hastened by increase in temperature. The fungal spores generally become more sensitive with age to the volatile toxin. When comparing onion pathogens and non-pathogens they found no strict negative correlation between pathogenicity to onion and sensitiveness to the toxins, which only indicates that other factors enter into the determination of the parasitic relation. Considering the onion parasites as between themselves there was evident a negative correlation between aggressiveness of parasitic attack and sensitiveness to the dissolved and volatile toxins. The presumption, however, is that as the parasites invade the tissue the host toxins, though attenuated by fungus enzymes, may possibly exert some retarding effect upon the invader. If this be the case, it is suggested that the host toxins may be one of the numerous factors which determine the degree of parasitism attained by a given parasite.

Vasudeva[5] gave an analysis of the factors responsible for the failure of *Monilia fructigena* to attack onion and *Botrytis Allii* to attack apple. He observed that the chief feature shown by spores of *Monilia*, when placed in wounds on onion, is their failure to germinate. This is due to the presence of a thermolabile substance which can be extracted with ether or chloroform. On the other hand, the failure of *B. Allii* to attack apple tissue is not due to any inhibitory or retarding action of apple juice. It could be made

to parasitise by adding to the inoculum a certain concentration of a nitrogenous substance. The effect of a nitrogenous compound in stimulating attack by *B. Allii* was found to run parallel with its effect in stimulating the secretion of the pectinase enzyme. He also found that by artificially ripening the apples, they become susceptible to *B. Allii* attack.

Chona[2] studied the enzymic behaviour of certain apple-attacking fungi (*Botrytis cinerea, Fusarium fructigenum*) with that of parasites on potato (*Pythium* sp., *Phytophthora erythroseptica*). Ordinarily the apple-attacking fungi did not attack potato and *vice versa* but he found that with the supply of an additional nitrogenous food a certain amount of such cross infection could be brought about. The pectinase activity could be retarded by pH concentration and by the action of certain plant extracts and chemicals, the retardation depending on the medium into which the enzyme was secreted.

Menon[3] has shown that the behaviour of the pectinase is modified by the nutrient medium in which the fungus is growing. According to him the nature of the nutrient medium modifies the capacity of a fungus to secrete pectinase. He assumes that certain substances are adsorbed from the nutrient medium and this adsorption modifies the properties of the pectinase.

Thornberry[4] has dealt with the pectinase activity of eight strains of *Fusarium* sp. from tobacco stems, two of *Sclerotium bataticola* (*Macrophomina phaseoli*), *Sclerotinia sclerotiorum, S. trifoliorum, Rhizoctonia* sp. from tobacco, three strains of *Thielaviopsis brasicola, Phytomonas* (*Bacterium*) *mori, P. tabaca* (*Bact. tabacum*), and *P. angulata* (*Bact. angulatum*). The determination of the pectinase activity was according to the method of Neuberg and Ostendorf (*Biochem. Z.*, ccxxix, p. 464, 1930). According to them extracts from pectase-active plant tissues hydrolyse the ester linkage of the half calcium salt of monomethyl tartaric acid. The ester being water-soluble and hydrolysable by pectase into soluble methyl alcohol and insoluble half calcium salt of tartaric acid, this method of determining pectase activity offers promise of utility for quantitative measurements based upon the precipitate formed. Thornberry by working with the above method observed that freshly isolated cultures of *Fusarium* sp. gave moderate hydrolysis, whereas little or no activity was shown by those that had undergone repeated subculturing since removal from their host. *S. sclerotiorum* and *S. trifoliorum* were only slightly active but considerable hydrolysis took place in the tubes inoculated with *M. phaseoli.* The tobacco *Rhizoctonia* gave negative results, while those obtained with *T. basicola* were variable.

The pectinase enzyme of the two fungi, *Sclerotium cepivorum* and *S. tuliparum*, was prepared from cultures on plugs as well as on flasks,

Only the enzyme excreted by the fungi was taken into account. The enzyme by the plug method was prepared by placing blocks of potato, turnip, etc., in boiling tubes having absorbent cotton wool, soaked with water, at the bottom. Such tubes were autoclaved, inoculated and incubated at 20° C. for different periods. The decayed portion as well as the fungal growth was removed and the juice squeezed, centrifuged and tested for pectinase. To obtain pectinase from flask cultures, 40 c.c. of medium were inoculated in 500 c.c. conical flasks and incubated at 20° C. for 10 to 20 days. The liquid was then filtered off and tested for pectinase. The test of activity was the usual one of the disintegration of potato discs (50 μ thick) as described by Brown.[1] For each experiment, as far as possible, potato discs were taken from the same potato so as to avoid tissue variation.

Preparations of the external enzyme were made in a standard manner from 15 days old plug cultures of potato, carrot, turnip, tulip and onion. These, when tested on standard potato discs, gave the activities shown diagrammatically in Fig. 1.

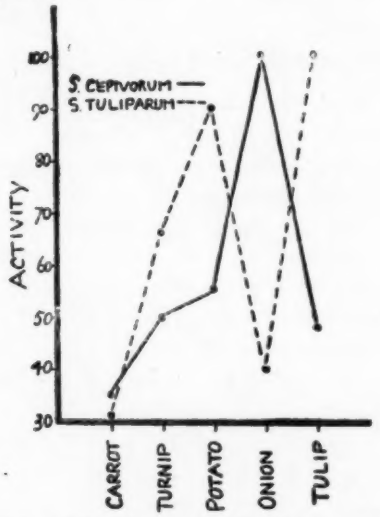

FIG. 1. Pectinase activity of *S. cepivorum* and *S. tuliparum* on different plug cultures.

From the figure it is clear that on media other than onion the activity of *S. cepivorum* is less than that of *S. tuliparum*. This applies also to tulip as a medium. On the other hand, the enzyme prepared from *S. cepivorum* on onion plugs is much stronger than that of *S. tuliparum* on this medium.

The corresponding diagram for the enzymatic extracts prepared from 15 days' old flask cultures is given in Fig. 2.

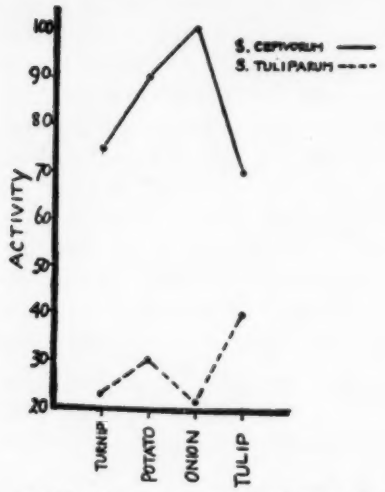

FIG. 2. Pectinase activity of *S. cepivorum*, *S. tuliparum* on flask cultures.

It will be noticed that here in all cases the pectinase activity of *S. cepivorum* is much stronger than that of *S. tuliparum*, but the tendency of the curves is the same as in the previous figure.

The enzyme from both the fungi was also obtained from 15 days' old flask cultures of synthetic and 20 per cent. tulip and onion extracts. The pectinase activity on different cultures from the two fungi are given in Table I.

TABLE I

Fungus	Pectinase activity			
	Richard's solution	Brown's starch	20% onion	20% tulip
S. cepivorum ..	100	71	83	80
S. tuliparum ..	100	83	71	86

The activities recorded in Figs. 1 and 2 have reference to potato discs as test material. A comparative study of potato, tulip and onion discs gave the data shown in Table II in which the times required for disintegration are recorded.

A comparison of columns 2 and 5 of this table shows that *S. cepivorum* produces a more active enzyme than *S. tuliparum* when onion plugs are used as media and that the converse applies when tulip plugs are used.

TABLE II

Fungus	Pectinase from onion plug			Pectinase from tulip plugs		
	Potato discs	Tulip discs	Onion discs	Potato discs	Tulip discs	Onion discs
S. cepivorum ..	40 min.	3 hrs. and 30 min.	1 hr. and 50 min.	60 min.	2 hrs. and 55 min.	2 hrs. and 10 min.
S. tuliparum ..	..	3 hrs.	3 hrs. and 40 min.	40 min.	2 hrs. and 25 min.	3 hrs. and 20 min.

Comparison of columns 2 and 3 shows that though the enzyme of *S. tuliparum* is only about half as active as that of *S. cepivorum* when tested on potato discs, it is fully more active when tested on tulip material. In other words tulip material is specifically more sensitive to the enzyme of *S. tuliparum.*

Similarly a comparison of columns 5 and 7 shows that the enzyme of *S. cepivorum* is specifically more active on onion material than is the enzyme of *S. tuliparum.*

Effect of various factors on Pectinase activity.—The data as regards the *effect of H-ion concentration* on the pectinase activity are given in Fig. 3.

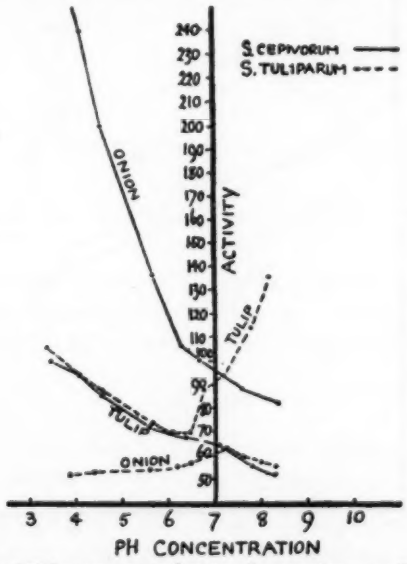

FIG. 3. Relation of H-ion concentration to the pectinase activity of *S. cepivorum* and *S. tuliparum.*

To obtain a range of pH, different amounts of N/20 HCl or NaOH were added. As a control to this experiment another range of pH was set up without the extracts which were tested for pectinase activity. Here it was found that within ten hours time there was no disintegration in the potato discs within 3·5–8·6 pH range. Therefore any effect shown within this range was due to the pectinase present. The pectinase activity of the extract without any added acid or alkali was taken to be 100.

A study of the above figure shows that there is a marked liking of *S. cepivorum* enzyme for acidity, especially the one from onion plugs. The curves for the preparation of *S. tuliparum* do not slope continuously to the right but either show no definite response to pH concentration at all or show a minimum of activity near the neutral point and a rather steep upward gradient on the alkaline side.

It has already been shown experimentally that the growth of *S. cepivorum* is fairly constant over a wide range (2·2 – 8·2) of H-ion concentration. A higher pH than 8·2 causes the growth rate to fall considerably. On the other hand the growth of *S. tuliparum* shows a well-defined optimum near the neutral point. The range of *S. tuliparum* in the alkali side is greater than that of *S. cepivorum* and conversely for the acid side.

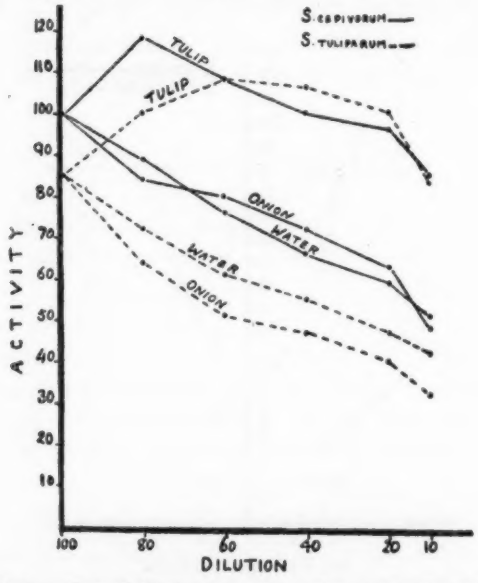

FIG. 4. The relative effect of dilution by water, onion and tulip juice on the pectinase activity from onion plugs.

It is interesting to recall in this connection that *S. cepivorum* is favoured in its growth, as stated above, by an acid reaction, so that to that extent the reactions of the fungus and of its enzyme are similar.

The relative retarding effects of various diluting substances (water, tulip or onion juice) are shown in Figs. 4 and 5. In the former the medium used was onion and in the latter tulip plugs. The cultures in both cases were three weeks old.

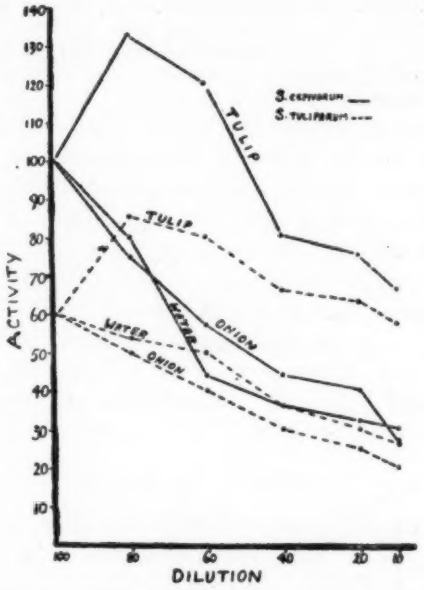

FIG. 5. The relative effect of dilution by water, onion and tulip juice on the pectinase activity from tulip plugs.

From both of these figures it is seen that certain dilutions by tulip juice instead of reducing considerably increase the activity while the presence of onion juice retards. The extracts of both fungi behaved similarly in this respect.

SUMMARY

1. *Sclerotium cepivorum* and *Sclerotium tuliparum* both were found to excrete pectinase enzyme on a variety of media.

2. *Sclerotium cepivorum* gave more active preparations of this enzyme when grown on onion than on tulip tissue, and the converse was true for *S. tuliparum*.

3. There was evidence that tulip tissue was specifically more sensitive to the enzyme of *S. tuliparum* than to that of *S. cepivorum* and conversely.

4. The enzyme prepared from *S. cepivorum* was more tolerant of acidity than that of *S. tuliparum*.

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STUDIES ON THE REFRACTIVE INDEX OF MILK

II. Some Factors Affecting the Refractive Index and Refractive Constant of Milk

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THE refractive index (R.I.) and Refractive Constant (K) of a large number of samples of cow and buffalo milk[1] and their normal limits of variation were given in an earlier paper (Rangappa, 1947). The samples in that experiment had been largely chosen at random from a big herd of about 400 animals without paying particular attention to any of the factors that are likely to affect the two values. But these values having been newly worked out for milk, the causes and extent of their variation under natural and routine conditions of animal management are to be looked for. With this object in view the effect of the more common factors like parturition, time of milking, quarter of udder, season, processing, storage of milk, etc., have been studied in this paper.

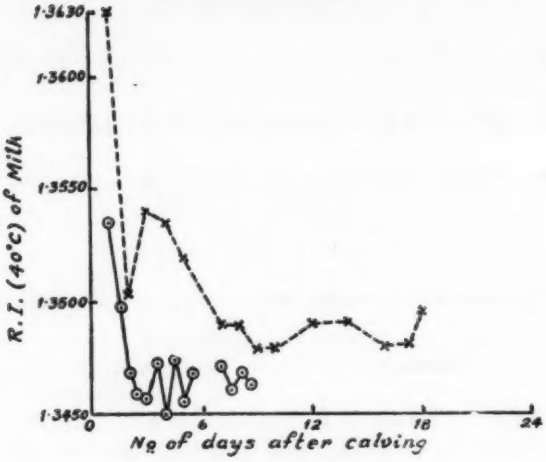
EXPERIMENTAL RESULTS

Milk samples were obtained from the same dairy farm which supplied samples for the last set of experiments (*loc. cit.*).

Effect of Calving.—Calving began this year in June and lasted through July up to about the end of August. This happens to fall in with the rainy season which continues intermittently up to November. The R.I. and K of a number of samples were followed through the colostral stage until the values became normal. Fig. 1 illustrates the effect of this factor.

The high initial values, it will be seen from the figure, come down to a steady normal in less than 5 days. It may be mentioned that the initial values fall in with the high S.N.F. contents of the colostrum. The refractive constant, also high initially, reaches normal levels a little sooner than the R.I.

Diurnal Variations.—Individual and bulk samples (from 15 to 25 animals) analysed over a week are illustrated in Fig. 2.



R. I. of Milk after calving

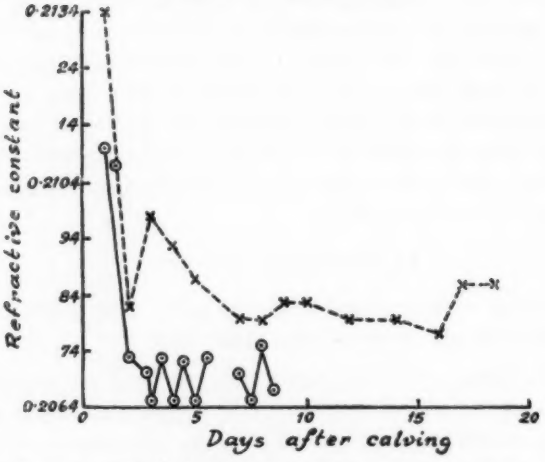


FIG. 1. Variations of Refractive Constant after calving

The figure shows appreciable fluctuations from day to day both of the R.I. and K of milk. But the variations in bulk samples are, as might be expected, not so wide as in individual samples. It is also to be noted that the range of variation of K is much less marked than that of R.I.

Effect of Time of Milking.—Fig. 3 illustrates the variations of the constants with the time of milking. The animals were milked at 7–30 in the morning and at about the same hour in the evening.

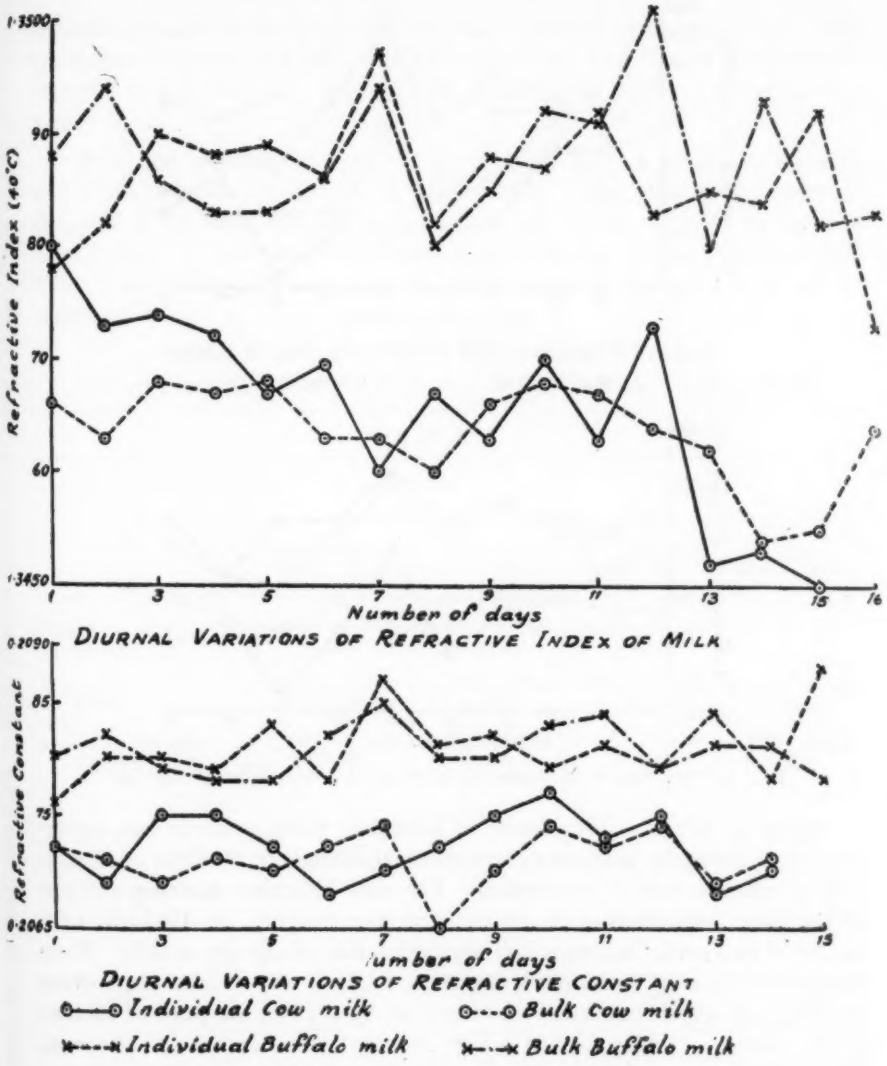
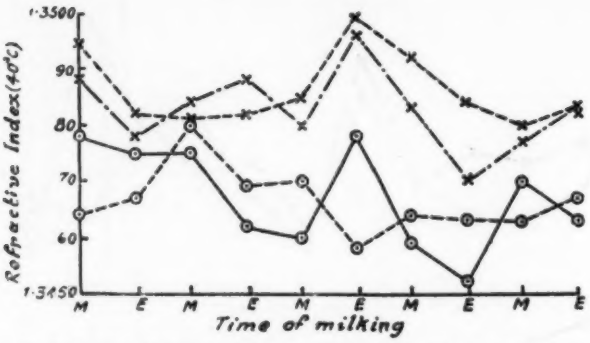


FIG. 2. These notations refer to all the figures in this paper

The figure shows that while the constants differ from morning to evening, the order of variation, like the differences from day to day, is unpredictable.



Variations in Refractive Index of Milk with Time of Milking
M = Morning E = Evening

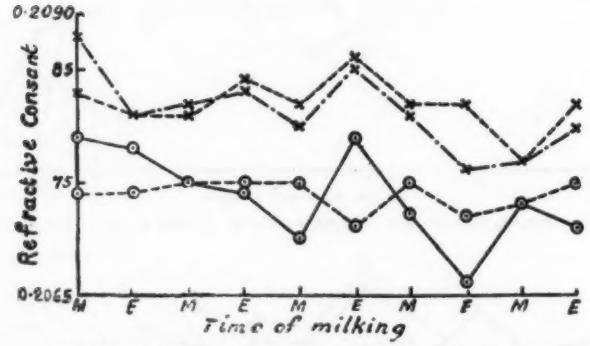


FIG. 3. Variations in Refractive Constant of Milk with Time of Milking

Effect of Season.—The climate of Bangalore being, more or less, temperate, throughout the year, no violent seasonal changes in the form of extreme cold or extreme heat is experienced. The only difference marking one part of the year from another is the wet and dry seasons. In 1945 the rains ceased in November marking the commencement of the dry season. From December onwards the animals began to feed on dry fodder (ragi straw) for roughage which continued up to end of April 1946, the peak of summer or dry season. In May the heavy S.W. monsoon rains started and continued intermittently up to the middle of June. During this time the animals were given partly green and partly dry fodder. This was followed by the N.E. monsoon beginning from the middle of July with frequent rains which lasted (unusually) through November and December. In the short interval between the two monsoons the cattle were fed for a few weeks on dry fodder, after which (August to December), they were given roughage made up half

of greens (maize stock, grass and lucerne) and half of chopped hay. The concentrates (groundnut cake, rice or wheat bran, and Bengal gram) at the rate of 1 lb. for every 3 lb. yield of milk remained the same throughout the year.

Bulk and individual samples were analysed twice a week, on Monday and Friday, from January to December 1946. The monthly averages of R.I. and K of these samples are given in Fig. 4. In addition to these, a large number of individual and bulk samples were also analysed every month. Table I gives the maximum and minimum values of R.I. and K of all the samples.

TABLE I

Seasonal variations in the R.I. and K of Cow and Buffalo Milk

Season	Individual		Bulk	
	R. I. (40° C.) Max. Min.	K Max. Min.	R. I. (40° C.) Max. Min.	K Max. Min.
COW				
January to April ..	1·3462 1·3443	0·2075 0·2065	1·3470 1·3449	.. ..
Average ..	1·3454	0·2068	1·3455	..
May to July ..	1·3470 1·3453	0·2074 0·2065	1·3470 1·3449	0·2074 0·2065
Average ..	1·3459	0·2069	1·3459	0·2070
August to December ..	1·3478 1·3450	0·2080 0·2064	1·3472 1·3458	0·2075 0·2066
Average ..	1·3466	0·2072	1·3463	0·2071
BUFFALO				
January to April ..	1·3488 1·3462	0·2086 0·2077	1·3488 1·3461	.. ..
Average ..	1·3474	0·2081	1·3477	..
May to July ..	1·3492 1·3462	0·2084 0·2076	1·3484 1·3462	0·2086 0·2076
Average ..	1·3479	0·2081	1·3474	0·2081
August to December ..	1·3497 1·3471	0·2088 0·2073	1·3501 1·3468	0·2088 0·2072
Average ..	1·3482	0·2080	1·3479	0·2079

It can be seen from the table and the figure that the rainy season, probably due to green feed, causes considerable change in the order of values of refractive index of milk. From May onwards (except for the interval between the monsoons in July) to December and January there is a marked upward shift in the limiting values of the refractive index of milk especially of cows, thus setting different limits for the wet and dry parts of the year. This is noticeable in both individual and bulk samples. The buffalo, how-

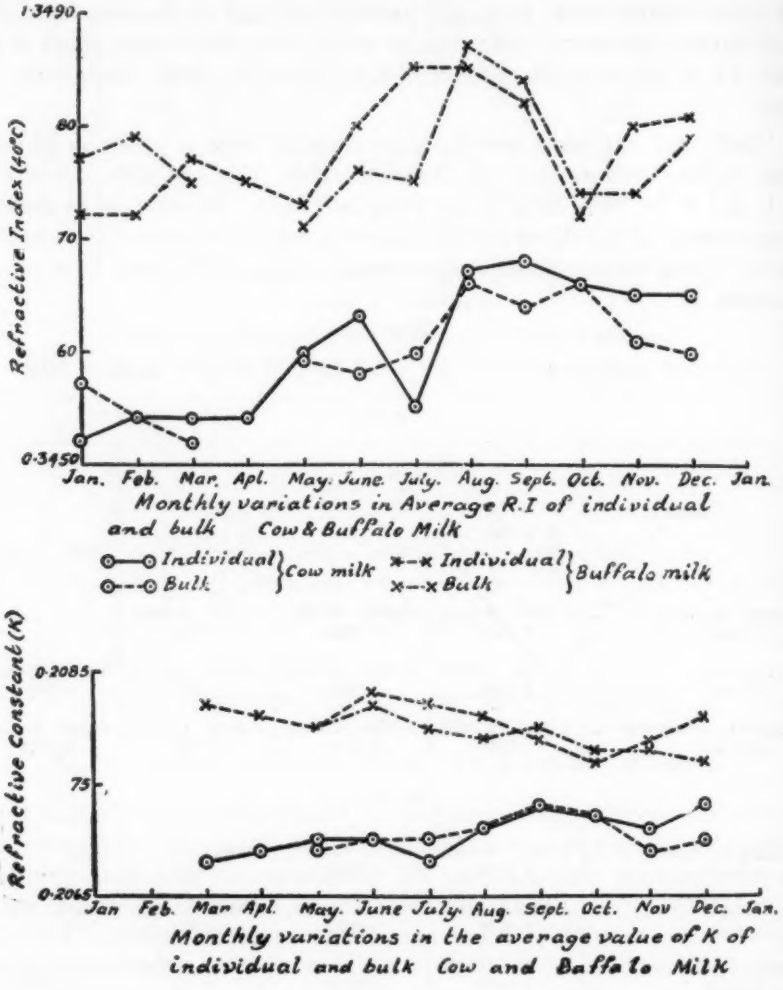


FIG. 4

ever, seems to be a little less susceptible to the changes in season than the cow. It should also be mentioned here that in normal years, when the rains usually end in November, the fall in R.I. might be expected to start earlier than during the current year. The refractive constant, on the other hand, runs more or less evenly within its narrow limits all through the year. This is obviously because the green feed which raises the R.I. also raises the density of milk.

Portions of a Milking.—A number of individual animals were milked, from fore milk to strippings, in 3 or 4 nearly equal parts, examined for R.I. and K, and the examination repeated after pooling all the yield from the animal. An example of the type of variation of the two values are given in Table II.

TABLE II

R.I. and K of Portions of a Milking

	COW		BUFFALO	
Portion	R. I. (40° C.)	K	R. I. (40° C.)	K
Fore milk ..	1·3467	0·2069	1·3477	0·2073
Middle ..	68	73	78	79
Final ..	67	78	78	82
Pooled milk ..	69	75	78	78

The table shows that only the refractive constant steadily rises from fore milk to strippings, and that the pooled milk gives values of K always within normal limits. This is accounted for by the falling density of the later portions of the milking (owing to the increasing fat content) while the R.I. remains practically the same.

Different Quarters of the Udder.—Milk collected separately from the four quarters of the udder have also been analysed. The results are given in Table III.

TABLE III

R.I. and K of Milk from Different Quarters of Udder

	COW		BUFFALOW	
Quarter	R. I. (40° C.)	K	R. I. (40° C.)	K
Left fore ..	1·3468	0·2067	1·3483	0·2079
Left hind ..	72	63	91	77
Right fore ..	69	68	87	76
Right hind ..	74	74	91	77
Bulked ..	70	75	88	83

The figures in the table show that appreciable differences do exist in the values of the constants of milk from the different quarters of the udder. But the differences are neither orderly nor predictable. This is to be expected as each quarter is a unit which functions independent of the others, The constants of pooled milk, however, lie within normal limits,

Storage of Milk.—In commercial practice there is usually a time lapse between milking and retailing the milk to the consumer. The effect of this factor on the refractive index was therefore studied. Milk samples were stored at room temperature (17°·5–25° C.) in conical flasks plugged with cotton-wool, the acidity and R.I. tested every few hours until the milk finally curdled. The graph connecting acidity with R.I. is given in Fig. 5.

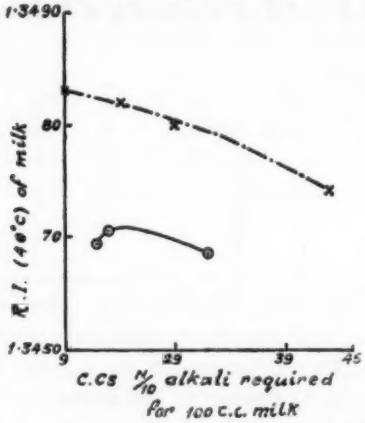


FIG. 5. Effect of Acidity on R. I. of Milk

It is apparent from the figure that the fall in R.I. is quite slow and gradual, there being a slight rise occasionally in the initial stages. Elsdon and Stubbs (1927) have also observed this fact with respect to milk serum. But the degree of or per cent. variation in the R.I. of milk with the rise in acidity before curdling compared to the variation in the R.I. of serum from sour milk or of stored serum is very inconsiderable. And the disadvantage referred to by the above authors arising from the rise in the value of R.I. of serum of old milk or of stored serum fails to arise in the case of the present method wherein it is impossible to determine the R.I. of soured milk for the simple reason that it has ceased to be milk owing to the precipitation of one of the major constituents, the casein, of milk. But this limitation of the applicability of this method to fresh milk can be overcome by adding the minimum amount of formaldehyde necessary to keep the milk from curdling. For it has been shown by Schultz and Wein (1913) that addition of formalin in such minute amounts causes no palpable difference in the refractive index of the milk serum.

In terms of time of storage of milk, it has been found in this experiment that there is no detectable change in the R.I. in the first 8 to 12 hours of storage.

It may be mentioned here that in all these experiments the limits of R.I. and K of cow and of buffalo milk remain characteristically distinct from one another.

Effect of Processing.—Boiling is the popular method of processing milk in India. Two litre samples of milk were therefore boiled in an open tinned-brass vessel over a kerosene stove with continuous agitation (to prevent the formation of skin and residue) up to a total of ten minutes after the milk commenced to boil. At intervals of a few minutes the reduction in volume, the R.I. of the processed milk and the densities were determined. Fig. 6

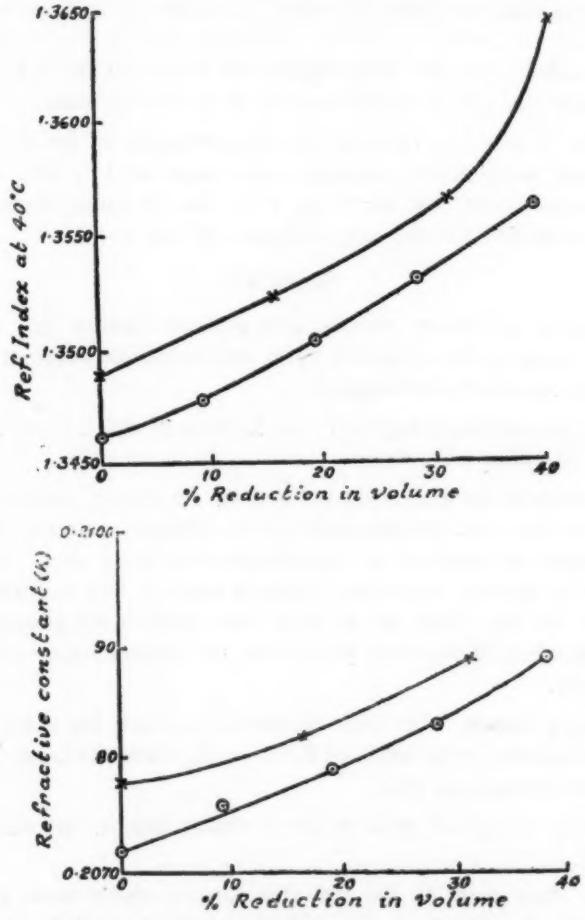


FIG. 6. Effect of Boiling on R. I. and K of Milk

represents the order of variation of R.I. and K, with respect to fall in volume of milk. The initial and final composition of the milk are given in Table IV.

TABLE IV

Effect of Boiling Milk on the Refractive Index and Constant of Milk

Milk	Density (20° C.)	Fat %	S. N. F. % (Calculated)	R. I. (40° C.)	K
Unprocessed ..	1·0292	5·2	9·05	1·3468	0·2072
Boiled for 10 minutes ..	1·0456	8·1	13·76	1·3563	0·2141

It is evident from the above figure and table that the R.I. and K rise steadily with the rise in concentration of processed milk.

Finally, in order to facilitate the determination of the R.I. at the prevailing room temperature, readings were taken at 15°, 20°, 27°, 35° and 45° C. It was found that for every 1° C. rise of temperature, the R.I. of milk falls by 0·00012 in the range between 20° and 45° C.

SUMMARY

A number of routine natural and artificial factors that are likely to affect the values of the refractive index and refractive constant of cow and buffalo milk have been investigated.

Colostrum exhibits a high R.I. and K, both of which reach normal levels in 3 to 5 days after parturition.

Differences in the two constants of milk occur from milking to milking, from day to day and between milks from different quarters of the udder. But the order of variation is unpredictable in every case. The different portions of a milking, however, exhibit a more or less uniform R.I. and a steady rise in the value of K from fore milk to strippings. But in all instances, pooling of the total yield from the animal restores the values to normal limits.

The rainy season, when lush pasture is available for cattle, appears to cause a marked rise in the limits of R.I. of milk, while the limits of K remain the same all through the year.

Rigorous boiling of milk causes a steady rise in the values of both R.I. and K.

All the data point to the fact that factors which cause a rise in the fat-free solids of milk also increase the measure of the R.I. The refractive

constant, on the other hand, remains within normal limits owing to a corresponding change in the density of milk under natural conditions of variation.

Acknowledgment

I wish to thank Mr. B. N. Banerjee and Prof. V. Subrahmanyan for their kind interest in these studies.

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THE NATURAL OCCURRENCE OF ERGOT IN SOUTH INDIA—III

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(Communicated by Rao Bahadur Dr. B. Viswanath, C.I.E., D.Sc., F.R.I.C., F.A.Sc.)

Since the publication of the earlier communications on the natural occurrence of ergot in South India (Thomas *et al.* I, II, 1945) more hosts affected by *Claviceps* have been discovered. Descriptions of the fungi on these hosts and the results of some inoculation experiments are recorded in this communication.

1. *Cynodon plectostachyum* Pilger.—This is a new grass recently introduced into Coimbatore by the Government Lecturing and Systematic Botanist for trial as a fodder. It was found to be severely infested by *Claviceps* during November 1945 to January 1946, and again in December 1946. The sphacelial form is conspicuous as white drops of sticky honey dew. A number of such drops are visible in a spike. The conidia are of two types. One kind of conidium is hyaline, oblong or reniform, measuring $15{\cdot}9 \times 6{\cdot}9$ $(11 - 8{\cdot}5 \times 3{\cdot}5 - 7{\cdot}4)\,\mu$ (Fig. 1). The second type is smaller more or less elliptical or sometimes subspherical, hyaline and measuring $9{\cdot}4 \times 7{\cdot}3$ $(7 - 11 \times 5{\cdot}6 - 9{\cdot}3)\,\mu$. The smaller type seems to be the secondary conidia formed by the germinating bigger conidia. The sclerotia usually develop in January. They are black, slightly bent, protruding beyond the glumes and measuring $3 - 5{\cdot}5 \times 1{\cdot}5$ mm.

This fungus closely resembles the one on *C. dactylon*. The conidial measurements and the shape and size of the sclerotia are almost identical on both the hosts and it is considered that both of them belong to the same species. A further comparison of the ergots occurring on different hosts brings out the close resemblance of the conidial stages on *Digitaria chinensis*, *D. wallichiana*, *Panicum maximum*, *Cynodon dactylon* and *C. plectostaehyum*. Spore suspensions of conidia from *P. maximum* were sprayed on open flowers of *C. dactylon*. Even after 15 days there was no sign of infection. But this does not necessarily mean the fungi on these two hosts are not the same species. In the genus *Claviceps* specialisation of parasitism exists and in the same species some strains do not pass on from one host to another (Atanasoff, 1920). However without the knowledge of the stromatal and perithecial characters it is unsafe to determine this species of *Claviceps*.

2. *Vetiveria Lawsoni* Blatt and MacC.—This is another grass under experimental cultivation in the botanic Garden at Coimbatore which exhibits severe infection by *Claviceps* in December. The conidial stage appears as sticky translucent drops which finally dry up into creamy white hard round masses outside the spikelets. Sometimes the fluid spreads over the glumes and

Fig. 1. Conidia of *Claviceps* on *Cynodon pheetostachyum.*
„ 2. „ *Paspalidium flavidum.*
„ 3. Sclerotia on *Paspalidium flavidum.*
„ 4. Conidia on *Cenchrus setigerus.*
„ 5. „ *Vetiveria lawsoni.*
„ 6. „ *Heteropogon contortus.*
„ 7. „ *Ischaemum pilosum.*
„ 8. „ *Urochloa panicoides.*

(All drawings of conidia × 500).

forms, on drying, white deposits over the surface of the affected and adjoining spikelets. From this white colouration the disease can be easily spotted. The conidia are hyaline, oblong with straight sides or constricted slightly in the middle. They measure $10{\cdot}2 \times 4{\cdot}2$ $(7{\cdot}4\text{–}11 \times 3{\cdot}7\text{–}5{\cdot}5)\,\mu$ (Fig. 5). The diseased spikelets are soon overgrown by *Cerebella* which arrests the

formation of sclerotia though it enables one to easily locate the affected spikelets. On dissecting open some of the spikelets, not overgrown by *Cerebella*, small black sclerotia 2–3·5 × 0·75–1 mm. in size can be seen between the glumes replacing the ovary. The base of the sclerotium is sometimes purple in colour.

3. *Ischaemum pilosum* Hack.—The honey dew is noticed as clear or white drops later turning brown outside the glumes. The conidia are hyaline and oblong with rounded ends. The contents are granular with the granules often grouped at the two ends. The conidia measure 11·4 × 5·0 (8·4–14·4 × 4·8–5·6) μ (Fig. 7). Here also *Cerebella* easily overgrows the fungal tissue and sclerotial formation is thus prevented. In some of the affected spikelets with no *Cerebella* infection, small dark sclerotia measuring 3 × 0·5 mm. were noticed inside the spikelets in the place of the ovary and completely enveloped by the glumes. The ergot on *Vetiveria lawsoni* and *Ischaemum pilosum* are identical and must be considered as belonging to the same species. They fall into the same group as those on *Themeda triandra*, *Ischaemum aristatum*, *Andropogon lividus* and *Cymbopogon flexuous* (Thomas *et al* 1945). Ajrekar (1920) has recorded a *Sphacelia* on *Ischaemum pilosum* but the spores are stated to be curved. Hence the fungus recorded now is quite different.

4. *Paspalidium flavidum* A Camus.—This is a common fodder grass found in many parts of the province. At Coimbatore it is affected by ergot in the months of November and December. The honey-dew protrudes as a sticky pearly drop from the spikelet. Later it may spread over the glumes and pedicels forming white crusts. The conidia are hyaline, lunulate and measure 16 × 5 (12·8–20·8 × 4·8–6·4) μ (Fig. 2). Sclerotia are formed. These are dark brown to black, curved, 4–5 × 1 mm. and projecting out from between the lemma and palea (Fig. 3).

5. *Urochloa panicoides* Beauv.—This is a good fodder grass common in all districts of the province. It is also affected by ergot at Coimbatore. The sphacelial stage develops in individual spikelets forming translucent to white drops exuding from the spikelets. Later, these harden into brown masses running over the glumes. Sometimes the whole spikelet is covered by a white deposit by means of which the affected spikelets can be easily recognised. The conidia are hyaline, fusoid to lunulate, measuring 15·4–5·1 (12·8–19 × 3·2–6·4) μ (Fig. 8). The sclerotia are small, brown, globose to oblong 1·5 × 0·5–0·75 mm. and occupying the position of the ovary between the lemma and palea. There is a close resemblance between the ergots on *Paspalidium flavidium*, *Urochloa panicoides*, *Urochloa reptans* and

Brachiaria distachya. The last named host was severely infested during December 1946 at Coimbatore. Thirumalachar (1945) has recorded a similar ergot on *B. distachya* from Mysore.

6. *Heteropogon contortus* Beauv.—Thomas *et al*. (1945) have recorded an ergot on this host having mainly triangular conidia. During December 1946 another type of sphacelial infection was noticed on this host besides the one recorded before. The honey-dew formation was more or less similar to the one noticed earlier but the conidia were different. They were hyaline oblong, with rounded ends, very rarely tending towards reniform and measured $15{\cdot}0 \times 5{\cdot}1$ ($11–19 \times 4{\cdot}8–6{\cdot}4$) μ (Fig. 6). The honey-dew soon dried into white or cream coloured masses projecting out of the spikelets.

Thirumalachar (1945) has also recorded from Mysore oblong conidia of the same size in sphacelial infection of this host. Thus this host appears to be susceptible to infection by two different types of *Sphacelia*.

7. *Cenchrus setigerus* Vahl.—This grass is common in all open places in this district. A widespread epiphytotic of *Sphacelia* was noticed on this grass from the month of November, 1946. The infection involved either some of the spikelets of the panicle or all the spikelets. A creamy white drop of fluid collected at the apex of the spikelet and flowed down the sides where it dried into white deposits clearly visible against the dark purple colour of the spikelet. The conidia were mainly lunulate sometimes fusoid, hyaline and measured $17{\cdot}9 \times 4{\cdot}8$ ($12{\cdot}8–26 \times 3{\cdot}2–6{\cdot}4$) μ (Fig. 4). The ovary and rarely the stamens also were enveloped in a compact hyphal mass. From the surface of this mass large members of conidiophores were developed in a closely packed folded hymenial layer. The formation of distinct sclerotia was not observed.

This resembles the *Sphacelia* recorded on *Cenchrus ciliaris* (Adyantayya, 1946) and *Pennisetum hohenackeri* (Thomas *et al*., 1945). Conidial suspensions of the *Sphacelia* on *C. setigerus* were sprayed on healthy blooming panicles of *C. ciliaris*, *C. setigerus*, *Brachiaria rumosa*, *Panicum maximum* and *Apluda aristata*. The inoculations were carried out during a spell of rainy weather and the experimental plants were kept inside glass cages or under bell jars for three days to provide favourable conditions for infection. On the ninth day a number of spikelets of *C. ciliaris*, *C. setigerus*, and *B. ramosa* were showing honey-dew formation. The controls and the other hosts were free. Thus the ergot passes from one host to another. The conidial characters also indicate that the same species is present on *C. ciliaris* *C. setigerus*, *B. ramosa* and *P. hohenackeri* (though inoculation experiments were not made on the last named host). The infection of the spikelets of

C. ciliaris is possible when inoculations are made when the anthers protrude or earlier. Six spikes were completely immersed in spore suspension for 2 minutes, three days after emergence and long before the flowers opened. In the course of ten days all the spikelets in the inoculated spikes were found infected with the honey-dew formation while the controls bloomed normally. Anthesis did not occur in the inoculated spikes.

Thirumalachar (1945) is of opinion that the ergot on *P. hohenackeri* "comes nearest to or is identical with *Claviceps microcephala*," judging from the colour of the stroma, size of ascospores and perithecia. But the conidia of this species are small and oval and measure 7–8 × 3–5 μ, while the conidia of the fungus on *P. hohenckeri* are bigger, lunulate, and measure 20·4 × 5·8 μ (Thomas *et al.*, 1945). Consequently it is evident that the ergot on *P. hohenackeri* is different from *C. microcephala*. Judging from the conidial characters the tentative grouping of the ergots suggested by Thomas *et al.* (1945–1) has to be slightly modified. In the first group in which curved and fusoid conidia are observed the two subdivisions may be modified as follows:—

Nature of conidia	Host plants
(1) Conidia of various shapes	
(*a*) Conidia reniform	*Cynodon dactylon*
	C. plectostachyum
	Digitaria chinensis
	D. wallichiana
	Panicum maximum
(*b*) Conidia lunulate or fusoid	*Urochloa reptans*
	U. panicoides
	Apluda aristata
	Cenchrus ciliaris
	C. setigerus
	Pennisetum hohenackeri
	Brachiaria ramosa
	B. distachya
	Paspalidium flavidum

8. *Sorghum* spp.—The occurrence of *Sphacelia sorghi* on many varieties of cultivated grain sorghums (*S. vulgare*, *S. aurra*, *S. Roxburghiana*, etc.) have been recorded from various parts of India, Burma and Africa. During this year the sugary disease was observed on a number of wild or exotic sorghums also at the Millets Breeding Station, Coimbatore, during December. The species that were involved are *S. halepense* Pers., *S. arundinaceum* Stapf.,

S. verticilliflorum Stapf., *S. nitens* (B. & P.) Snow., *S. caffrorum* Beauv., and *S. membranaceum* Chiov. In all these pearly drops were seen exuding from the spikelets. These later dried into white deposits sometimes connecting together the contacting spikelets. The ground underneath the affected plants was bespattered with white spots. The conidia in all cases were alike being oblong with rounded ends and slightly constricted in the middle measuring on an average $16 \times 7\,\mu$ (12-19 $\times$ 5-8). The size, shape of conidia, and the symptoms of infection are similar on all the hosts and agree with those of *Sphacelia sorghi* McRae. Sclerotial formation was not in evidence on any of the hosts.

Summary

Six new hosts of *Claviceps* have been recorded and the fungal characters on these hosts are described. These fungi fall into one or the other of the groups previously recorded by Thomas *et al.* (1945) for the ergots occurring in South India. A slight modification of the grouping adopted by Thomas *et al.* (1945–1) in classifying the ergots by the conidial characters, has been made. Wild and exotic species of sorghum were infected by *Sphacelia sorghi*.

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PHYTOPHTHORA PALMIVORA BUTLER CAUSING A SEEDLING BLIGHT OF *HIBISCUS ESCULENTUS* L.*

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(Communicated by Rao Bahadur Dr. B. V. Nath, F.A.SC., C.I.E., D.SC., F.R.I.C.)

While raising seedlings of *Hibiscus esculentus* L. for inoculation experiments with a species of *Pythium* recently isolated from that host, it was noticed that there was severe collar rot and seedling blight in many of the pots. Most seedlings were attacked while still in the cotyledonary or four-leaved stage and infected plants soon wilted, fell over due to rotting at the collar, and died (Pl. VII, Fig. 1). Infection was very severe during rainy spells followed by cloudy days resulting in losses ranging from 85 to 90 per cent. During one week in particular when there was very heavy rain for three days followed by overcast weather all the 180 seedlings in 25 pots were killed. Examination of blighted seedlings showed extensive rotting of the collar region often followed by stem and root rot (Pl. VII, Fig. 2).

The causal organism was isolated by using bits of surface sterilised tissue taken from the margin of the rotted portions and small bits of rotted stems and roots. Out of a total of 20 isolations, 17 yielded pure growths of a species of *Phytophthora*. In the remaining three instances *Rhizoctonia solani* was present along with *Phytophthora*. However, as *Phytophthora* was present in all cases and as 17 out of 20 isolations yielded pure growths of this fungus, it was assumed that this was the causal agent and that *R. solani* was only a secondary parasite. Inoculations carried out later with pure cultures confirmed this conclusion.

Isolates of this fungus grew well on most culture media, growth being especially luxuriant on oatmeal, frenchbean and carrot agars. Of these three, oatmeal was found to be the best and was used for maintenance of pure cultures. The organism develops copious coarse aerial mycelium on the culture media mentioned above and both sporangia and chlamydospores are produced in abundance in cultures over 22 days old.

The sporangia (Pl. VI, B.C.) were sub-spherical or limoniform, papillate, terminal or intercalary and measured on an average $35 \times 25\,\mu$ (the range being $20-40 \times 18-35\,\mu$). The zoospores were reniform and laterally

* Contribution from the Mycology Section, Agricultural Research Institute, Coimbatore.

biciliate measuring 8 to 12 μ by 6 to 8 μ while swimming (Pl. VI, I*a*, *b*, *c*, *d*) and 8 to 10 μ when encysted (Pl. VI, J, *a*). After encystment they germinated by producing one to three germ tubes (Pl. VI, J, *b*, *c*, *d*, *e*).

The chlamydospores (Pl. VI, D, E, F, G) were spherical or sub-spherical, terminal or intercalary and ranged from 20 to 35 μ in diameter (average 28·5 μ).

The measurements of sporangia and chlamydospores given above fall within the range given by other workers for *Phytophthora palmivora*, a species which occurs commonly in South India. As the present isolate did not form oospores in pure culture even after three months, paired cultures were made with known plus and minus strains (Thomas *et al.*, 1947) of *P. palmivora* available in the stock culture collection at the Mycology Section, Coimbatore, in an attempt to induce oospore formation. The results of these trials are shown in the table below:

	Isolate with which the *H. esculentus* Phytophthora was grown in paired culture	Result
1.	*Phytophthora palmivora* (Plus strain) isolated from *Areca catechu*, S. Kanara	Oospores formed in 4 days
2.	do (Plus strain) isolated from tomato, Coimbatore	do 36 hrs.*
3.	do (Plus strain) isolated from *Colocasia antiquorum*, S. Kanara	do 3 days
4.	do (Plus strain) isolated from *Clerodendron infortunatum*, S. Kanara	do 3 days
5.	do (Plus strain) supplied by Dr. Uppal, Bombay	do 3 days
6.	do (Plus strain) isolated from *Cyphomandra betacea*, Burliar	do 36 hrs. *
7.	do (Minus strain) supplied by Dr. Uppal, Bombay	No oospores formed
8.	do (Minus strain) isolated from *Spondias mangifera*, S. Kanara	do
9.	do (Minus strain) isolated from *Carica papaya*, Coimbatore	do

* The rapidity with which oospores are formed in these two instances is due to the freshness of the isolates (*cf.* Thomas *et al.* 1947).

These results show that the *H. esculentus* isolate is a minus strain of *P. palmivora*.

The oogonium is spherical or sub-spherical with a fairly slender stalk encompassed by the persistent amphigynous antheridium (Pl. VI, K, L, M, N, O, P). While young, the oogonia had hyaline walls and granular contents which became brownish after fertilization and the differentiation of the oospore. The oospores were usually spherical, thick-walled and not quite filling the oogonium. When ripe, the walls of the oogonia and

oospores were golden brown to dark brown in colour. Oogonia ranged from 20 to 40 μ (av. 30·5 μ) in diameter and oospores from 18 to 30 μ (av. 23·5 μ). These sizes are well within the range given by previous workers for oospores and oogonia formed in paired cultures of *P. palmivora* (Ashby, 1929; Davidson, 1934; Gadd, 1924; Lester-smith, 1927; Marudarajan, 1941; Narasimhan, 1930 ; Thomas *et al.*, 1947; Thompson, 1924 and Venkatarayan, 1932).

Inoculations were carried out with this fungus on *H. esculentus* seedlings using the terminal bud inoculation technique described by Wiant and Tucker (1940) for *Phytophthora capsici* Leonian. A severe die-back and rotting of the younger portions resulted, the infected plants being killed in 10 to 15 days.

In addition, soil inoculations were also carried out. The procedure adopted was as follows: Good viable seeds of *H. esculentus* were treated with an organo-mercury compound (usually Agrosan—GN) and then sown in sterilized soil in glazed pots. Sterile distilled water was used in subsequent watering to obviate the possibility of water-borne contamination. When the seedlings were about 10 to 12 cm. tall, a hole was made in the soil in the centre of the pot, roughly 8 cm. away from the seedlings, care being taken to see that the roots of the young plants were not injured and bits of agar cultures of the fungus introduced; the hole was then covered up and the pot kept covered by a sterilized bell-jar. Suitable controls were also set up simultaneously. Infection was observed in the inoculated pots 7 to 10 days after inoculation with typical symptoms of collar-rot, wilting and tendency to fall over. Infected plants were killed in 13 to 17 days. All controls remained healthy and unaffected. The pathogen was successfully reisolated from diseased seedlings.

Young and old fruits of *H. esculentus* were also inoculated with small bits of agar cultures after surface sterilization and kept covered with a sterilized bell-jar. The fruits took infection readily and rotted within five days. In all cases the controls remained healthy and unaffected.

A survey of literature shows that so far only one member of the Pythiaceæ—*Pythium deBaryanum* Hesse—has been recorded on *Hibiscus esculentus* L. (Ramos, 1926). No species of *Phytophthora* has till now been reported to attack this plant though *P. parasitica* Dastur and *P. palmivora* have been recorded on *Hibiscus sabdariffa* (Tucker, 1933; McRae, 1932; Thompson, 1933), *H. sabdariffa* var. *altissima* (Tucker, 1933; Hector, 1931; Kar and Saha, 1943), and *H. manihot* (Tasugi and Ikeda, 1939) and

H. cannabinos (Muller and Van Eek, 1939). This appears to be the first record of *P. palmivora* on *Hibiscus esculentus*.

The writer wishes to acknowledge his indebtedness to Mr. K. M. Thomas, B.A., M.Sc., D.I.C., Government Mycologist, and Mr. T. S. Ramakrishnan, M.A., Assistant Mycologist, for their kind guidance and help. The work was done with a grant given by the Indian Council of Agricultural Research for the study of Pythiaceous fungi.

Summary

Phytophthora palmivora Butler was isolated from blighted seedlings of *Hibiscus esculentus* L. Inoculation experiments proved its pathogenicity. A study of its cultural and sexual behaviour showed that it was a minus strain, not forming oospores in pure culture and forming them only when grown in paired cultures with complementary (plus) strains of the same species.

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Explanation of Plates

Plate VI

A. Vegetative hypha.

B and C. Terminal and intercalary sporangia.

D to G. Terminal and intercalary chlamydospores.

H. Liberation of zoospores.

I. Free swimming zoospores.

J. Encysted (*a*) and germinating (*b–e*) zoospores.

K to P. Oogonia and oospores of the *Hibiscus esculentus* Phytophthora with other isolates of *P. palmivora.*

K. *H. esculentus* × Tomato.
L. do. × *Cyphomandra betacea.*
M. do. × *Areca catechu* (S. Kanara).
N. do. × do. (Dr. Uppal's isolate).
O. do. × *Clerodendron infortunatum.*
P. do. × *Colocasia antiquorum.*

Note.—All figures were drawn with the aid of an Abbe camera lucida at table level. The magnification in the case of figures A–H is × 540 and figures I — P × 765.

Plate VII

Photographs of diseased *H. esculentus* seedlings.

Fig. 1. Diseased (A) and healthy (B) potted seedlings.

Fig. 2. Seedlings showing different stages of infection; (A) Start of infection, note lesion at the collar region; (B) and (C) more advanced, about half the stem is rotted in (C); (D) plant completely rotted and dead.

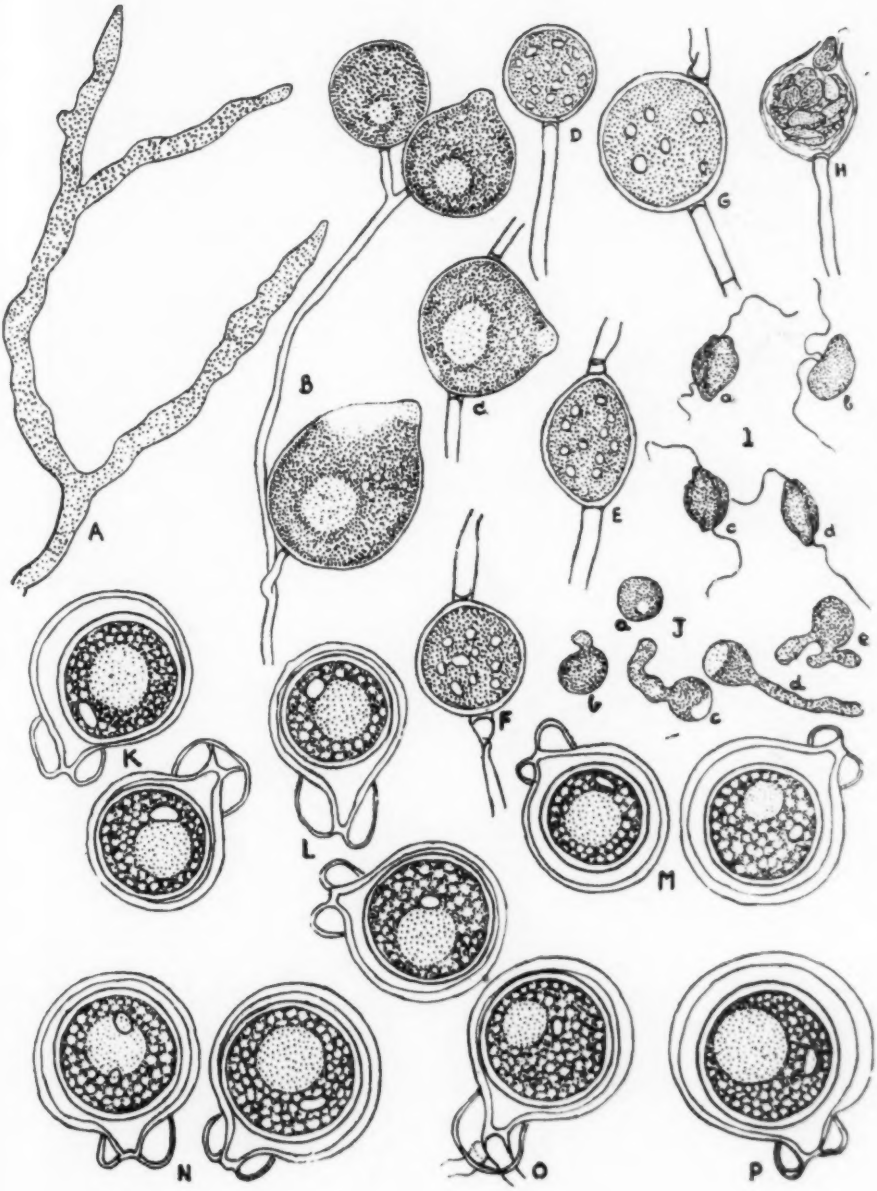
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FIG. 1

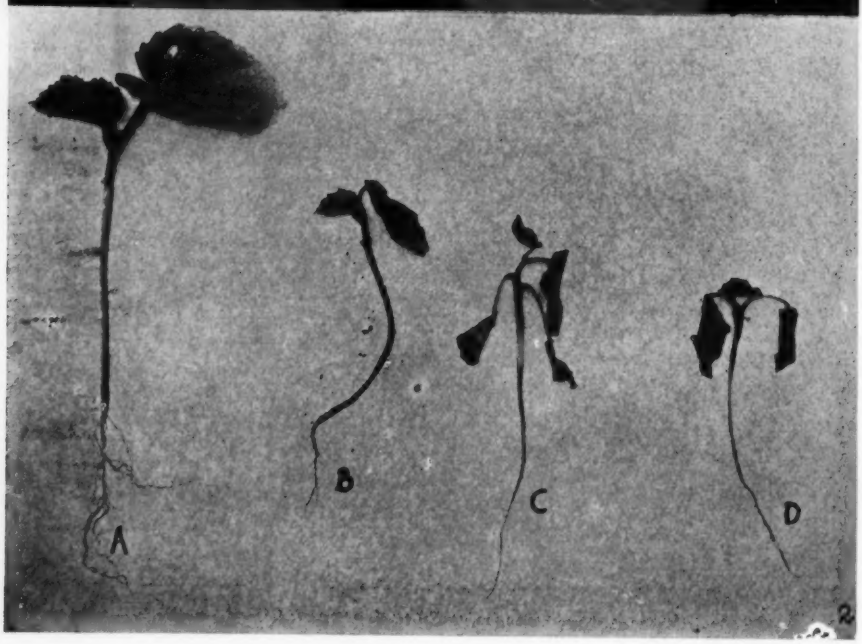

FIG. 2

STUDIES IN THE GENUS PHYTOPHTHORA

I. Oospore Formation and Taxonomy of *Phytophthora palmivora* Butler

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The development of the sexual bodies in *Phytophthora palmivora*, Butl. has been investigated by a number of workers from time to time. Aided by these and other physiological reactions the status of this species has been revised every now and then and modified to take in more than one species created by later workers in its fold. The arguments put forward by the revisionists have been varied.

Coleman (1907) was the first to report on oospore formation in cultures of *P. omnivora* var. *arecæ* Colem. [(later named *P. arecæ* (Coleman) Pethy]. Rosenbaum (1917) obtained oospores of *P. arecæ* in cultures received from Coleman. But other workers have not been able to obtain these sexual bodies in single strain cultures. Narasimhan (1930) has put forward the explanation that the cultures sent to Rosenbaum were probably not a single strain but a mixed one and that this might be responsible for the formation of oospores. The same explanation is applicable for the development of oospores in Coleman's cultures also. McRae (1917) has described the production of oospores in single strain cultures of *P. meadii* McRae. But this ability is shortlived and other workers to whom cultures of this fungus had been supplied found that oospores did not develop in the cultures. The isolate now studied also does not produce oospores in single strain cultures. Ashby (1922) noted that in paired cultures of *P. palmivora* and *P. faberi* Maub. (coconut and cacao strains) oospores developed. Later (1929) he continued the studies on the development of sexual bodies in paired cultures of *Phytophthora* and has recorded their formation in paired cultures of isolates from coconut and cotton; citrus and coconut; rubber and cacao; and coconut (India) and coconut (Jamaica). As a result of these studies and with isolates from other sources he recognised that the isolates he had, could be placed into two groups, the members of one group forming oospores when mixed with members of the other. Adopting Gadd's (1925) group nomenclature, he arranged the isolates into the 'cacao' and 'rubber'

groups. In the 'cacao' group were isolates from cacao, coconut (India), papaya, *Vanda* and *Cattleya* and in the 'rubber' group isolates from coconut (Jamaica and Philippines), Citrus, *Hevea*, *Dendrobium* and *Odontodenia*. Lester-Smith (1927) grew three isolates of *P. faberi* Maubl. in paired cultures and obtained oospores. In combination with *P. parasitica* Dast. also *P. faberi* formed oospores. Paired cultures of *P. parasitica* strains and *P. nicotianæ* Br. de Haan, also produced oospores. Gadd (1925) made a comparative study of the strains of *Phytophthora* isolated from cacao, papaya, *Hevea*, *Dendrobium*, *Odontodenia* and breadfruit in Ceylon. He found that these strains produced oospores in paired cultures; the isolates from cacao and papaya behaved as 'plus' strains and others as 'minus' strains. His later studies (1927) have confirmed his earlier conclusions but he found that the isolates from areca in Ceylon did not form oospores with isolates of *P. faberi* strains. Thompson (1929) obtained eight isolates of *Phytophthora* from *Hevea brasiliensis* which he classified into *P. palmivora*, *P. meadii*, and *P. heveæ* Thompson. These formed oospores in paired cultures with other strains (coconut and roselle) of *P. palmivora* and *P. parasitica*. Narasimhan (1930) studied oospore formation in paired cultures of isolates from *Areca*, *Santalum album* L., *Loranthus longiflorus* Desv., *Jatropha curcas* L., *Bryophyllum calycium* Salisb., *Artocarpus integrifolia* L., *Colocasia antiquorum* Schott and *Ficus hispida* L. He found that in paired cultures the isolates from *Loranthus* and *Areca* developed oospores with the isolates from *Santalum* and *Jatropha*. Leonian (1931) studied the behaviour of 85 cultures of *Phytophthora*. He found that 48 of them were heterothallic equally divided into males and females while the remainder were classified into inconstant forms exhibiting heterothallism, and neutral behaviour. The cultures he tested included *P. palmivora*, *P. faberi*, *P. parasitica*, *P. terrestris* Sherb., *P. manoana* Sid. and *P. nicotianæ*. Venkatarayan (1932) was able to obtain oospores in paired culture of two isolates of *P. arecæ* and *P. palmivora*. Uppal and Desai (1939) obtained oospores in paired cultures of two isolates of *P. arecæ* from Bombay Province. Marudarajan (1941) investigated the formation of oospores in six isolates from *Areca*, *Hevea*, coconut, palmyra and *Cacao* and agreed with Gadd in the existence of two groups. He continued his investigations with two isolates from *Clerodendron infortunatum* L. and *Spondias mangifera* Willd., each belonging to one of the above groups.

Materials and Methods

The availability in the stock cultures of the Government Mycologist, Coimbatore, of a large number of isolates of this genus and of *P. palmivora* as it is now understood was taken advantage of to study their sexual behaviour

under controlled cultural conditions. The list of isolates used in these studies with the accepted identifications as far as they were known at the commencement of these studies is given in Table I.

TABLE I

List of the isolates of Phytophthora used in the study of paired cultures

S. No.	Host	Part affected	Pathogen	Locality	Source from which the isolate was obtained
1	*Agave wightii*, Dr. and Pr.	Leaf	*Phytophthora parasitica* Dast.	Coimbatore	Local isolation from Madras Province
2-6	*Areca catechu* L. (Four isolates)	Fruit	*P. arecæ* (Colem.) Pethy.	South Kanara	do
	(One isolate)	do	do	Mysore	Mr. M. J. Narasimhan, Bangalore
	do	do	*P. arecae* (Strain Tyagli.	North Kanara, Bombay	Dr B. N. Uppal, Poona
	do	do	*P. arecae* (Strain Nilekani)	do	do
7	*Artocarpus incisa* L.	do	*P. palmivora* Butl.	South Kanara	Local isolation from Madras Province
8	*A. integrifolia* L.	do	*P. arecae* (Colem.) Pethy.	do	do
9	*Borassus flabellifer* L.	Bud	*P. palmivora* Butl.	Malabar	do
10	*Citrus nobilis* Lou. (I)	Leaf and fruit	do	do	do
11	*Citrus sinensis* Osbeck (11)	Base of stem	*P. palmivora* Butl.	Kistna	Local isolation from Madras Province
12	*Clerodendron infortunatum* L.	Leaf	P. sp.	South Kanara	do
13	*Cocos nucifera* L.	Bud	*P. palmivora* Butl.	Malabar	do
14	*Colocasia antiquorum* Schott.	Leaf	P. sp.	South Kanara	do
15	*Hevea brasiliensis* M. Agg.	Leaf and fruit	*P. meadii* McRae	Cochin State	do
16	*Lycopersicum esculentum* Mill.	Fruit	*P. arecae* (Colem) Pethy.	Coimbatore	do
17	*Nicotiana tabacum* L.	Stem	*P. parasitica* var. *nicotianae*	Salem	do
18	*Piper betle* L. (3 isolates)	do	*P. palmivora* Butl.	Chingleput and Tanjore	do
19	*Spondias mangifera* Willd.	Fruit	do	South Kanara	do
20	*Theobroma cacao* L.	do	*P. faberi* Maubl.	Ceylon	Government Mycologist, Ceylon
21	*Jatropha curcas* L.	do	P. sp.	South Kanara	Local isolation from Madras Province

All the isolates were pure strains and non-oospore forming at the time when the study was commenced. Some of them have been reported to have produced oospores in single strain culture but at the time of the studies no

oospores could be detected in any of the cultures. The isolates from agave, breadfruit and *Hevea* come under this class. Paired cultures were grown in petri-dishes or agar slants. In petri-dishes, quadrants were marked on the dishes by cutting out furrows 2–3 mm. wide in the media through the centre of the dish at right angles. The two strains used in paired cultures were inoculated on adjacent quadrants so that oospore formation if any could be detected easily in the clear furrows where the two growths meet when examining the undersurface of the dish under the low power of the microscope. On agar slants in tubes, the two strains were placed side by side on one edge of the slant half way down its length so that the periodic examination of the tube under the low power of the microscope was facilitated. Except when otherwise mentioned, the cultures were grown on oat agar media at laboratory temperature.

Results of Experiments

Experiment I.—At the outset the two isolates from *Areca* which were obtained from Dr. Uppal were grown together; oospore development was observed on the fifth day in the furrows between adjacent quadrants. In the course of ten days numerous oospores had developed in the zones of both the strains besides those formed in the furrow. Dark lines or zones representing the areas of oospore formation described by Narasimhan (1930) were, however, absent.

Experiment II.—The next step was to find out the sexual behaviour of the different isolates from *Areca* available at Coimbatore. Each of the five isolates, four from South Kanara and one from Mysore, was grown together with each of the two Bombay strains. It was found that all these five isolates formed oospores with the Tyagli strain but not with the Nilekani strain. This explains why Marudarajan (1941) failed to get oospores in mixed cultures of the areca strains from this province. Obviously all of them happened to belong to the same group and further studies revealed that these isolates corresponded with the Nilekani strain from Bombay.

Experiment III.—In a third series of experiments a large number of the isolates of *Phytophthora* available at Coimbatore and originally isolated from a variety of hosts were grown in paired cultures with the two Bombay strains of *P. arecæ* with the following results (Table II).

It is clear from Table II that all the isolates used in this experiment fall into two distinct groups one forming oospores with the Tyagli strain and the other with the Nilekani strain.

Experiment IV.—Paired cultures were then made of various permutations and combinations of all isolates available at Coimbatore other than

TABLE II

Results of paired culture studies made with two Bombay isolates of P. arecæ

Isolate	Result of pairing with	
	Tyagli strain	Nilekani strain
Agave ..	No oospores	Oospores formed
Breadfruit ..	do	do
Citrus II ..	do	do
Coconut ..	do	do
Hevea ..	do	do
Jatropha ..	do	do
Palmyra ..	do	do
Cocoa ..	do	do
Spondias ..	do	do
Areca ..	Oospores formed	No oospores
Betel vine (3 isolates)	do	do
Citrus I ..	do	do
Clerodendron ..	do	do
Jak ..	do	do
Tomato ..	do	do

the two Bombay isolates of *P. arecæ* supplied by Dr. Uppal and observations on the formation of oospores were recorded.

The results are given below:—

TABLE III

Results of paired culture studies among original collections of Phytophthora available at Coimbatore

Isolates	Areca	Betel Vine	Cit. I	*Clerodendron*	Jak	Tomato	Agave	Breadfruit	Cocoa	Cit. II	Coconut	*Colocasia*	*Hevea*	*Jatropha*	*Spondias*	Palmyra	Tobacco
Areca ..	0	0	0	0	0	0	×	..	×	×	×	0	×	×	×	×	×
Betel vine ..	0	0	0	0	0	0	..	..	..	..	×	..	×	..	×	..	..
Citrus I ..	0	0	0	..	..	0	..	..	×	×	×	..	×	×	×	×	..
Clerodendron ..	0	0	..	0	0	0	×	..	×	..	×	0	×	..	×	..	..
Jak ..	0	0	..	0	0	0	×	..	..	×	×	..	×	×	×	×	..
Tomato ..	0	0	0	0	0	..	×	×	×	..	×	..	×	×	×	×	×
Agave ..	×	..	..	×	×	×	0	0	0	..	0	..	0	0	0	0	..
Breadfruit ..	×	×	×	×	×	×	0	0	..	..	0	×	..	..	0	..	..
Cocoa ..	×	..	×	×	×	×	0	..	..	0	0	..	0	0	..	0	..
Citrus II ..	×	..	×	..	×	..	..	..	0	..	0	..	0	0	..	0	..
Coconut ..	×	×	×	×	×	×	0	0	0	0	..	..	0	0	0	0	0
Colocasia ..	0	..	..	0	..	..	..	..	..	..	..	..	..	..	×	..	..
Hevea ..	×	×	×	×	×	×	0	..	0	0	0	..	0	0	0	0	..
Jatropha ..	×	..	×	..	×	×	0	..	0	0	0	..	0	0	0	0	..
Palmyra ..	×	..	×	..	×	×	0	..	0	0	0	..	0	0	0	0	0
Tobacco ..	×	..	..	..	..	×	..	..	..	..	0	..	..	..	..	0	0
Spondias ..	×	×	×	×	×	×	0	0	..	..	0	×	0	0	0	0	..

Remarks.—The isolate from breadfruit died soon after the commencement of this experiment; it could not be utilised for further study of paired cultures.

0 = No Oospores. × = Oospores formed. .. = Combinations not tried.

The behaviour of these isolates has been consistent and in conformity with the results obtained in previous experiments. The isolates fall into two sexually distinct types. The members of one group invariably form oospores in paired cultures with members of the other group but not amongst themselves.

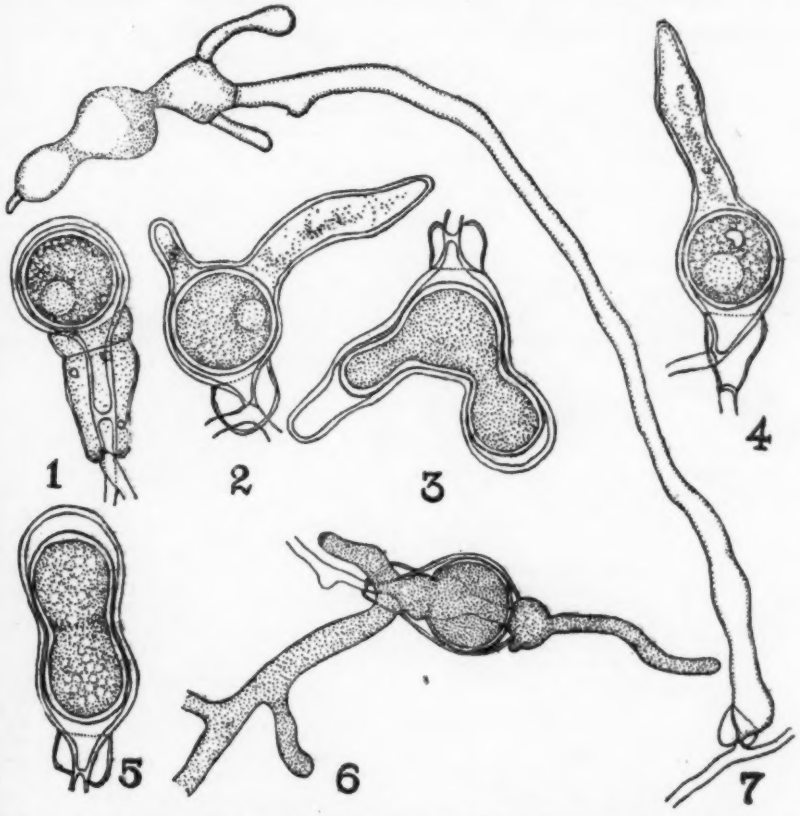
Morphology of the Sexual Organs

The oogonia are spherical, thick-walled, light yellow to yellowish brown in colour, persistent and always with one amphigynous antheridium at the base. Sometimes double antheridia may be seen one above the other (Text-Fig. 1). The antheridia and oogonia always developed on different hyphæ. No instance of the two organs developing on the same hypha was seen. The oogonial wall exhibits variations. In some combinations all the oogonia were smooth, but in others like *Citrus* I × *Jatropha*, *Citrus* I × *Citrus* II, *Citrus* I × palmyra and *Citrus* I × coconut some of the oogonia had thicker walls which were not as clear cut as in others but possessed a rough outline due to uneven thickening of the outer surface (Figs. O, P, Q). This feature has been noted by Gadd (1924) in some of the paired cultures of isolates from Ceylon. Tompkins and Tucker (1937) observed a thick brown crystalline encrustation on the wall of the oogonium produced in paired cultures of *P. capsici* Leon. Consequently the exact limits of the oospore and oogonia could not be determined while taking measurements. With the addition of concentrated solution of potassium hydroxide the encrustation disappeared and the walls of the oogonia and oospores became clear. The thickening of the oogonial wall noticed in some of the cultures under study however did not react in a similar manner when potassium hydroxide solution was added.

The oospores are spherical, thickwalled and light yellow to yellowish-brown or reddish-brown in colour. They may either completely fill the oogonial cavity or there may be some space between the two walls. In all paired cultures varying proportions of the two kinds can be observed.

In some of the cultures (*e.g.*, *Citrus* I × *Jatropha*) some of the later formed oogonia and oospores were peculiar. The oogonium was elongated, irregularly swollen and sometimes developing one or more branches (Text-Figs. 2–5, 7). Usually only one oospore was found in such oogonia but this occupied only a portion of the oogonium while the rest of the cavity

was either empty or filled with vacuolate hyaline or yellowish protoplasmic contents. Oospores also exhibited lobulation in some cases. Some of the oogonia were very much elongated and branched and usually these did not contain oospores. They resembled empty, swollen, elongated and branched hyphæ with scanty or disintegrated contents. The presence of the amphigynous empty antheridium at the base distinguished these structures from vegetative hyphæ and showed them as malformed oogonia. Leonian (1931) has observed similar structures in a culture of *P. palmivora*. But he describes them as germinating oospores and the round bodies inside some of them as secondary oospores. But the writers do not agree with this. These structures represent abnormal proliferating oogonia. The



TEXT-FIGS. 1–6.—Sexual bodies from Citrus I X *Jatropha*. 1. Double antheridia. 2–5. Abnormal oogonia with differently shaped oospores. 6. Germinating oospore. 7. An abnormal oogonium showing peculiar growth and branching (× 680).

germination of the oospore can be easily distinguished by the presence of the germ tube which bursts through the wall of the oospore and later through the wall of the oogonium and grows out (Text-Fig. 6). But in most of the cases this was not evident and the oogonium alone had elongated and developed branches. When the oospore germinates the oogonium does not elongate. The sketches given by Leonian (1931) also do not bear out his statement. They can be regarded only as abnormal oogonia. Those oogonia which have not been fertilised probably resume vegetative activity and develop into branched structures of limited growth.

In tubes and petri-dishes the sexual bodies develop on the medium or are submerged; they are also formed on the sides of the glass in tubes and in the clear space between the quadrants in petri-dishes. The first formed oospores are usually at the junction of the growths from the two isolates.

TABLE IV

Measurements of oogonia and oospores as recorded by various workers

Author	Species or pair of isolates	Oogonia		Oospores	
		Range μ	Mean μ	Range μ	Mean μ
Rosenbaum ..	*P. arecae* ..			23–44	32·4
Coleman ..	do ..			23–36	
Ashby ..	Cacao+Coconut 3 ..	22–35	28·6	17·8–27·8	23·1
	Cacao+Cotton boll ..	do	29·0	17·8–28·6	23·1
	Cacao+*P. parasitica* (castor) ..	25–33	28·8	20·0–25·3	23·6
	Coconut (Jamaica)+Coconut (India)			26–39	31·8
	Hevea+Cacao (Hevea Zone) ..			22–33	27·6
	(Cacao Zone) ..			20–30	25·0
	P. meadii+*P. arecae* ..	24–34	28·5	20–28	24·0
	P. meadii (Malaya)+*P. arecae* ..	24–35	29	23–28	25·3
McRae ..	*P. meadii* ..	24–35	32	16–32·8	25·0
Gadd ..	Cacao+*Odontodenia* ..			19–25	22·1
				21–28	23·8
	Cacao+rubber ..			20–28	23·7
	Cacao+Breadfruit ..			22–27	24·0
	Papaw fruit+*Odontodenia* ..			17–28	23·4
Narasimhan ..	Areca+*Santalum* ..			30–31	
	Areca+*Jatropha* ..			26–27	
	Santalum+*Loranthus* ..			30–31	
	Loranthus+*Jatropha* ..			29–30	
Venkatarayan ..	*Aleurites*+areca (fruit) ..		27·8		23·04
	do + do (top rot) ..		25·8		22·2
	Areca (top rot)+*Santalum* ..		28·9		25·5
	Areca (fruit) + do ..		32·6		28·1
Marudarajan ..	*P. arecae*+*P. meadii* ..	28–42	35·7	24·5–38·5	31·5
	do +Palmyra ..	28–40	33·6	do	29·5
	do +Coconut ..	28–42	36·0	do	30·8
	do +Citrus ..	24·5–33·3	29·4	22·8–31·5	27·3
	do +Cacao ..	18·3–33·3	28·0	17·5–29·8	24·5

Later they may be observed in other portions also. In some combinations oospores are formed in plenty while in others they are few. This difference in the intensity of formation of sexual bodies may be due to the fact that the isolates were originally brought into culture at different periods and consequently varied in the number of generations they had passed through in subcultures on agar media.

The size of the oogonium and the oospore exhibited wide variations. The measurements of the oogonia and oospores obtained by previous workers are given in Table IV.

These measurements were compared with those of the sexual bodies produced in the paired cultures under study. One hundred measurements were made in each case. The sexual bodies were taken from paired cultures within ten to fifteen days after inoculation (Table V).

TABLE V

Measurements of sexual bodies obtained from different combinations

Serial No.	Isolates grown in paired cultures	Diameter			
		Oogonia		Oospores	
		Range in μ	Mean μ	Range in μ	Mean μ
1	*Areca* (Nilekani)+Palmyra ..	23–41	30·0	18·6–30·0	24·4
2	*Areca* (Nilekani)+*Jatropha* ..	21·7–40·3	28·6	14·0–31·0	21·8
3	*Areca* (Nilekani)+*Spondias* ..	14·0–33·3	23·9	10·5–28·0	17·5
4	Jak+Palmyra ..	20·2–27·9	25·1	15·5–21·7	19·5
5	Jak+*Jatropha* ..	21·7–34·1	27·1	15·5–26·5	20·9
6	do+*Citrus* II ..	21·7–34·1	29·5	14·0–27·9	23·0
7	do+*Hevea* ..	20·2–34·1	27·2	15·5–27·9	20·8
8	do+*Agave* ..	21·7–31·0	25·8	15·5–24·8	19·9
9	do+*Spondias* ..	23·3–34·0	28·4	18·6–27·9	22·5
10	do+*Areca* (Tyagali) ..	24·8–34·4	29·8	20·2–27·9	12·3
11	*Citrus* I +*Spondias* ..	21·7–34·1	27·1	15·5–27·9	21·6
12	do +*Citrus* II ..	24·8–37·2	30·6	18·6–31·0	24·7
13	do +Palmyra ..	21·7–34·1	28·1	17·1–26·4	21·6
14	do +*Jatropha* ..	24·8–37·2	29·1	18·6–31·0	23·0
15	*Clerodendron*+*Hevea* ..	21·7–32·6	27·5	17·1–24·8	21·1
16	*Clerodendron*+*Spondias* ..	23·3–34·1	29·4	18·6–31·0	24·0
17	Betel vine I+*Spondias* ..	21·7–32·6	27·5	18·6–27·9	21·7
18	Betal vine II+*Spondias* ..	24·8–37·2	30·0	18·6–31·0	23·8
19	Betel vine+*Areca* (Tyagali) ..	24·8–36·5	28·4	15·5–31·0	21·9
20	Citrus I+Tyagali ..	21·7–30·0	27·5	15·5–24·8	21·0
21	*Clerodendron*+*Areca* (Tyagali) ..	24·8–35·7	30·5	17·1–27·9	23·4
22	*Areca* (Kanara)+*Spondias* ..	21·7–34·1	28·4	18·6–27·9	22·4
23	*Colocasia*+*Spondias* ..	21·7–34·1	27·5	15·5–24·8	21·9
24	Tomato+Tobacco ..	20·2–27·9	24·1	15·5–21·7	18·0
25	Tomato+*Jatropha* ..	21·7–31·0	25·1	18·6–27·9	21·9
26	Tomato+*Citrus* II ..	21·7–34·1	29·1	18·6–27·9	24·8
27	Tomato+Coconut ..	23·3–34·1	27·7	20·2–29·6	23·4
28	Breadfruit (alone) (suspected to be mixed)	20·0–28·5	25·0	16·5–24·5	20·6
29	Tomato+*Hevea* ..	24·8–34·1	28·1	20·2–29·6	24·1

The mean diameter of the oospores varies from 17·5 to 24·4μ and lies within the range obtained by other workers. The wide variation in size of the sexual bodies observed in these studies and by other workers goes to show that this character is highly variable and plastic and that no reliance can be placed on this for taxonomic purposes. However the ability to form the oospores in paired culture brings out the specific relationship of the complementary isolates.

Some Physiological Studies

The influence of medium on oospore formation.—It has been stated by previous workers that certain media favoured the formation of oospores in mixed cultures while others did not. This factor differs with isolates. Thus Tucker (1931) found that certain isolates of *P. parasitica* produced large numbers of oospores in lima bean and oat-meal agars and few or none on cornmeal agar, while still others developed more oospores on cornmeal agar and a smaller number on oat-meal agar. The investigations on paired cultures recorded here were carried out on oat agar which was found to be quite satisfactory. Leonian (1931) also found that oat agar was the most suitable. Two complementary strains, *viz.*, *Citrus* I and *Citrus* II were grown on oat, frenchbean and maize agars. The reaction of the media was adjusted to pH 5·6 in each case. The growth of the fungi was very luxuriant on oat and frenchbean agars and less profuse on maize agar. Oospores were formed in all cases but were more numerous in french-bean and oat agars than in maize agar. Tucker's observations only show the possibility that different races have preference to particular media for growth and reproduction.

Liquid oat-extract was prepared by boiling 50 gm. of powdered oat grains in a litre of water for one hour and then filtered through cottonwool. After filtering, the extract was autoclaved for 20 minutes at 15 lbs. pressure. In this medium two strains were grown for 15 days after which the medium was filtered through Chamberland filters under aseptic conditions. Five and ten cubic centimetres of the filtrates were mixed with 10 c.c. of melted oat agar medium which was then poured into plates. After the agar had set, the plates were inoculated with the complementary strain. Even after 30 days' growth oospores were not formed. This indicates that a strain does not secrete any extra-cellular substance into the medium to stimulate oospore formation in its complementary strain. Further work on these lines is in progress.

Temperature and oospore formation.—Ashby (1929) has recorded that if paired cultures are maintained at 23° C. (or 20°–25° C.) prompt development

of oospores takes place. Other workers also have experienced that exposure to lower temperatures or maintenance of cultures in ice-chests is conducive to oospore formation. Marudarajan (1941) observed oospore formation to be good at 20° C. In the course of the present investigation it was observed that oospores did not develop in the paired cultures started in the months of March, April and May when the laboratory temperatures varied between 28° C. and 31° C. But in July and August and from October to January the paired cultures readily produced oospores when the laboratory temperature was below 26° C. Paired cultures kept inside a controlled temperature cabinet in which the temperature varied from 8°–10° C. failed to develop oospores. Very low temperatures evidently do not favour the development of oospores in this tropical species.

Age of isolates and oospore formation.—In fungi, it is common experience that the intensity of sporulation gradually diminishes as the isolate is kept on for a large number of generations on agar media and may even disappear eventually. This behaviour is often seen in *Phytophthora* especially with regard to oospore formation. Paired cultures of fresh isolates of complementary strains produce oospores quite readily and in large numbers in 3–8 days depending on the distance separating the inocula of the two strains. But after several generations of sub-cultures the capacity to form oospores decreases in some strains until it is finally lost. For instance the Nilekani strain on *Areca* obtained from Bombay used to form large numbers of oospores with its complementary strains as mentioned earlier. But at the time of writing, *i.e.*, two years after its arrival, it does not form oospores with the isolates with which it was forming oospores before. It has become neutral.

The isolate from *Spondias* is another good example of the waning of the capacity to form oospores with ageing. A fresh isolate formed oospores with all the complementary isolates in four days. Another which had been isolated two years ago produced sexual bodies in combination with the same complementary strain. But the development was incomplete. Antheridia and oogonia were formed but mature oospores did not develop. The oogonia had grown through the antheridia and assumed the normal size and shape after emergence but later the contents disintegrated. Six months later, even this phenomenon did not occur in the combinations. Thus there has been a gradual decline of the sexual capacity of the isolate. This phenomenon is attributable either to the senescence of the isolate through successive subculturing on media for a long period or to formation of indistinguishable dissociants which were neutral or had lost their sexuality. These dissociants

have possibly been carried over in the transfers and thus the change might have occurred. Further experiments are necessary to decide the correctness of this view.

Discussion

The study of the formation of oospores in *Phytophthora* has been an interesting subject for investigation and several workers have been on this problem, though the last word has not yet been written. Oospore formation is influenced by various factors such as the temperature at which the organism is grown, the medium on which it is grown, the age of the isolates and lastly the innate character of the isolate itself. There are some species in which the sexual bodies have not been recorded yet. The development of the sexual bodies in the *Phytophthora palmivora* group has been investigated by different authors and divergent views have been expressed about the causes leading to this phenomenon. One school represented by Ashby (1928-29) and Lester-Smith (1927) is of the opinion that the oospore formation is brought about by some sort of biochemical stimulation of one strain by the other. Lester-Smith states that the production of oospores " in mixed cultures is due to the influence of one vegetation on the other acting through its effect on the medium or on certain constituents of the medium ". Gadd (1924), Thompson (1929), Narasimhan (1930), Leonian (1931), Venkatarayan (1932) and Uppal and Desai (1939) on the other hand believe in the heterothallic nature of the isolates of this species. Some prefer to call the isolates ' Plus ' and ' Minus ' strains while Narasimhan and Leonian who have traced the origin of the hyphæ producing the antheridia and oogonia, call the isolates male and female. Tucker (1931) is not convinced of the heterothallic nature of the isolates.

The present investigations carried out with 25 isolates of *Phytophthora*, the bulk of which were obtained from this province and a few from outside the province, have shown that all of them fall into two distinct groups based on their capacity to produce oospores in paired cultures. The isolates of one group form oospores when mixed with isolates from the other. Different combinations of the members of the two groups have been made and the results have been consistent throughout. In the light of present knowledge this behaviour can only be attributed to heterothallism within the same species.

It has moreover been found that in all cases the oogonia and antheridia are borne on different hyphæ and never on the same hypha. This again is an indirect evidence of heterothallism. Narasimhan (1930) and Leonian (1931) have claimed to have traced the antheridia and oogonia to different thalli, which is a direct proof of heterothallism of the isolates studied.

It has also been found that some isolates have gradually lost their sexuality in course of time when grown on media. The deterioration of the capacity for oospore-formation seems to be attributable to gradual loss of the sexual vigour of the strains through continued growth on agar media.

The behaviour of some of the isolates which formed oospores in single strain cultures originally but later failed to produce them, is intriguing. This can be explained away in two ways. It is possible that the original culture was itself a mixed one as might be expected when the fungus is isolated by tissue cultures and the original host had been infected by both the strains. An observation made by the writers in 1946, favours this view. In 1946 a fresh isolate was obtained from breadfruit by tissue culture. In the first generation abundant oospores were formed. From this culture single hyphal tips were transferred to agar slants. Oospores failed to form in these secondary cultures indicating that the original isolate was mixed. But McRae (1917) recorded oospores in single sporangial isolates of *P. meadii.* It has been noticed by several investigators that the cultures supplied to them from Coimbatore did not develop oospores. Even in India the same phenomenon was experienced. McRae had observed oospores on *Hevea* fruits also. This could be explained on the assumption that the strain was originally homothallic but during the growth of the cultures on agar media for a number of generations dissociation took place and the loss of one sexual factor resulted therefrom. It is, however, interesting to note that the isolates from *Agave*, *Hevea* and *breadfruit*, which were originally reported to be forming oospores in single strain cultures and have now become non-oospore-forming, fall into the same sexual thallus group which produces oospores in paired cultures with individuals of the same complementary group. No isolate belonging to the opposite group isolated in this province has ever been known to form oospores in single strain cultures. Leonian (1931) obtained seven dissociants from a culture of *P. parasitica.* Of these six behaved as females and one as a male. He has also obtained other dissociants which could be termed neutral since they failed to form oospores with either of the male or female isolates. Even in the heterothallic strains under study the sex vigour has been lost owing to long culturing on agar media or formation of neutral dissociants. Thus members of the *P. palmivora* group behave as homothallic, heterothallic or neutral strains though with continued growth on agar media many of the strains may become neutral. This change is observable in both groups of complementary strains. Therefore, for the correct identification of the isolates fresh cultures are essential.

These investigations have been helpful in deciding the taxonomic relationships of the isolates. The isolates studied have been variously classified at present. Tucker (1933) and Leonian (1934) have suggested certain revisions of the classification of *Phytophthora* species. Tucker has merged together *P. palmivora*, *P. arecæ*, *P. faberi* and *P. meadii* into one species under *P. palmivora*. Leonian believes that *P. mexicana* Hots. and Hart., *P. parasitica*, *P. parasitica* var. *rhei* God., *P. parasitica* var. *nicotianæ* Tucker, *P. terrestris* Sherb., *P. melongenæ* Saw. and *P. symmetrica* Sid. also should be brought under *P. palmivora*. The isolates under investigation are usually classified as follows: *P. palmivora* on coconut, palmyra, citrus and betel vine; *P. arecæ* on arecanut and tomato; *P. faberi* on cocoa; *P. meadii* on *Hevea*; *P. parasitica* var. *nicotianæ* on tobacco; *P. sp.* (not determined) on *Clerodendron*, breadfruit, *Spondias*, *Jatropha*, Jak and *Colocasia* and *P. parasitica* on Agave.

The basis for specific differentiation has been morphological features of the hyphæ, sporangia, chlamydospores and oospores, when formed. Pathogenicity has also been utilized for separating species. Studies on this genus have shown (Tucker, 1931 and Leonian, 1934) that the morphological characters of the mycelium, sporangia and chlamydospores are so plastic as to be of little use in specific differentiation. Leonian (1934) says that "pathogenicity is of still less value, the shape and size of the chlamydospores altogether useless and that of the sporangia not much better in the taxonomy of species of *Phytophthora*".

The work now recorded has shown that all the isolates under study produce oospores when grown mixed with complementary isolates. All the oospores formed in the various combinations are of the same type and the measurements fall within the range recorded for oospores produced from complementary strains occurring on the same host, *e.g.*, *Areca* (Tyagali) × *Areca* (Nilekani) 14·0–31·0 μ. This feature coupled with the readiness with which oospores are formed in the paired cultures of these isolates brings out the close specific relationship of these isolates. There can be no question of regarding these oospores as of hybrid origin between different species because no constant differences can be made out between these either in the size of the oospores, the nature of the antheridia or any other important character. All these isolates, therefore, fall under one species, *viz.*, *P. palmivora* Butler.

This species has a wide host range. Not less than sixteen species of host plants have so far been recorded from S. India. It is heterothallic but the present state of our knowledge suggests the possibility of some isolations

being homothallic. Two distinct sexual strains—the plus and the minus—are seen and the collections in our possession are classified under the two heads as follows:

Plus	Minus
Areca (Nilekani)	*Areca* (Tyagali)
Betel vine	Palmyra
Citrus I	Coconut
Clerodendron	Cacao
Colocasia	Rubber
Jak	Breadfruit
Tomato	*Spondias*
	Agave
	Tobacco
	Citrus II
	Jatropha

The grouping of the isolates into the " Cacao " and " Rubber " groups adopted by Gadd and followed by Ashby is rather confusing. The same host has been found to be affected by both the strains. For example, the " Cacao " isolate from Ceylon really behaves like an isolate belonging to the " Rubber " group. Ashby has also found that different isolates from cacao and coconut may fall into different groups. Therefore, the naming of the groups according to the host is misleading.

Uppal and Desai (1939) have obtained two complementary isolates Tyagali and Nilekani—form the same host, *viz.*, *Areca*. Tyagali behaves like the isolates from palmyra and coconut in Madras; Butler (1910) has recorded that *P. palmivora* affects arecanuts causing bud-rot. It is possible that the Tyagali strain represents the coconut strain (minus) which has become parasitic on *Areca* in that locality. Coconut is also infected by both the strains. *Citrus* in India is also parasitised by both. When such mixed or combined infections occur in nature on the same host plants, there is every possibility of oospores developing as has been recorded in breadfruit and *Hevea* rubber. This represents one of the methods of ' over-summering ' of the fungus under tropical conditions obtaining in South India. Whether sexual reproduction gives rise to new races is a matter for further investigation.

The plant pathologist has to consider the significance of these results. These facts bring out the necessity for vigilance on his part concerning the occurrence of *Phytophthora palmivora* on a variety of hosts some economically important and others of no importance. Inasmuch as this species

has a wide host range, the passage from one host to another is easy under favourable conditions. Further, the part played by the non-crop-hosts in the survival of the pathogen, the formation of sexual bodies when the same host becomes infected by the two sexual strains and the possible production of new strains as a result of sexual reproduction cannot be overruled. The parasitism of *P. palmivora* being by no means specialised, every record of this species on any host has to be considered as a source of potential danger to the crop plants known to serve as hosts of this species in the locality.

Acknowledgements

We are grateful to Dr. B. N. Uppal, Plant Pathologist, Bombay, and Mr. M. J. Narasimhan, Director of Agriculture, Mysore, for having kindly sent the cultures of strains of *P. arecæ*.

Summary

The formation of oospores in paired cultures of twenty-five isolates of *Phytophthora* was studied. These isolates fall into two main groups—the " plus " and the " minus " and the members of one group form oospores when paired with members of the other group. Some of the isolates were found to lose their sexual capacity with continued cultivation on agar media. Fresh isolates form oospores quickly with complementary strains.

All these isolates belong to *P. palmivora*, Butl. The other species of *Phytophthora, viz., P. arecæ, P. meadii, P. faberi* and *P. parasitica* var. *nicotianæ*—are to be merged in *P. palmivora* as they are found to be morphologically similar and do not exhibit any constant and reliable differences from *P. palmivora* and readily form oospores when paired with it. This species is heterothallic; but homothallism has been reported to have been noticed in some isolates.

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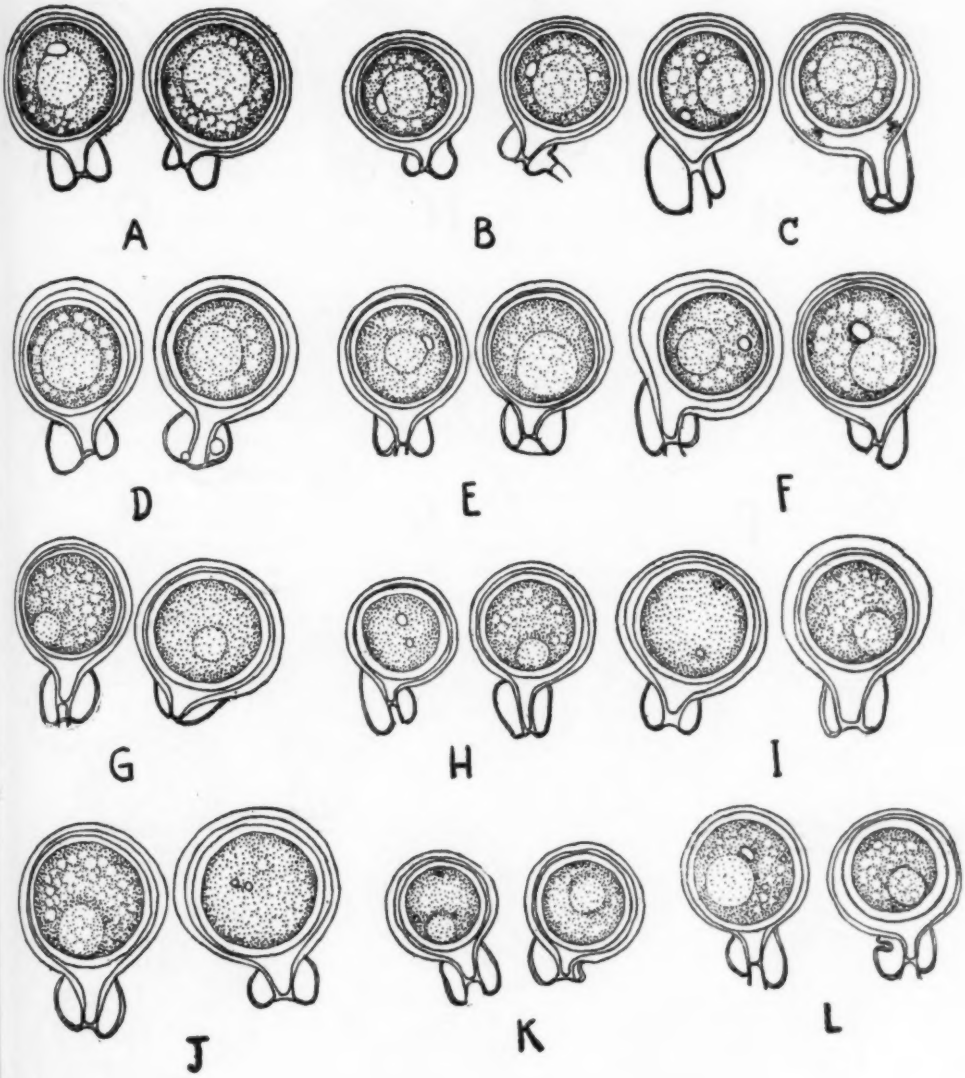
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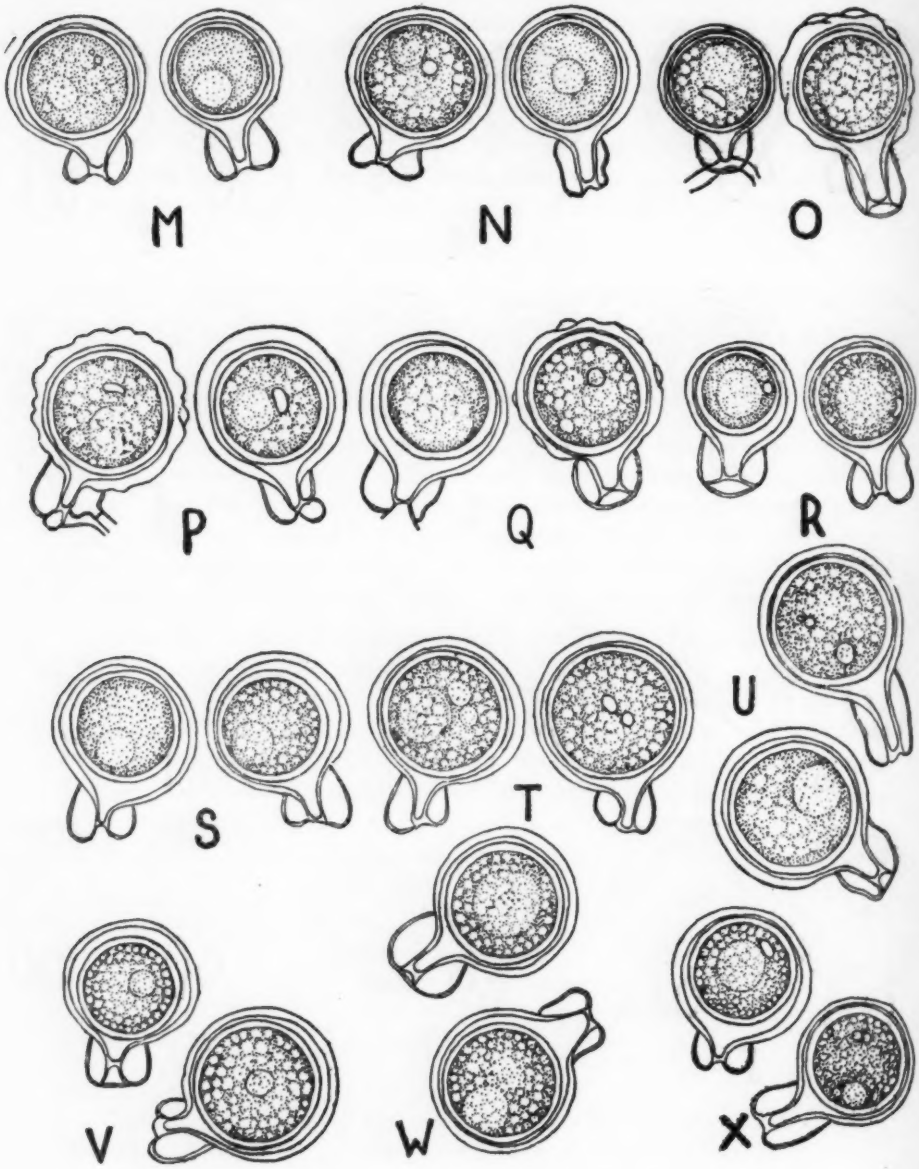
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Explanations of Plates

All figures were drawn with the aid of an Abbe camera lucida at a uniform magnification of × 680.

Plates VIII and IX : Sexual bodies produced in paired cultures of 'plus' and 'minus' strains of *Phytophthora palmivora* Butler.

Plate VIII

A. Jak × Jatropha
B. Jak × Palmyra
C. Jak × Hevea
D. Jak × Citrus II
E. Jak × Agave
F. Jak × Spondias
G. Tomato × Citrus II
H. Tomato × Jatropha
I. Tomato × Cocoa
J. Tomato × Hevea
K. Tomato × Tobacco
L. Tomato × Agave

Plate IX

M. Tomato × Coconut
N. Citrus I × Spondias
O. Citrus I × Jatropha
P. Citrus I × Palmyra
Q. Citrus I × Citrus II
R. Breadfruit (alone)
S. Clerodendron × Hevea
T. Clerodendron × Spondias
U. Betel vine I × Spondias
V. Betel vine II × Spondias
W. Colocasia × Spondias
X. Areca × Spondias

EMBRYOGENY OF *ISOTOMA LONGIFLORA* PRESL.

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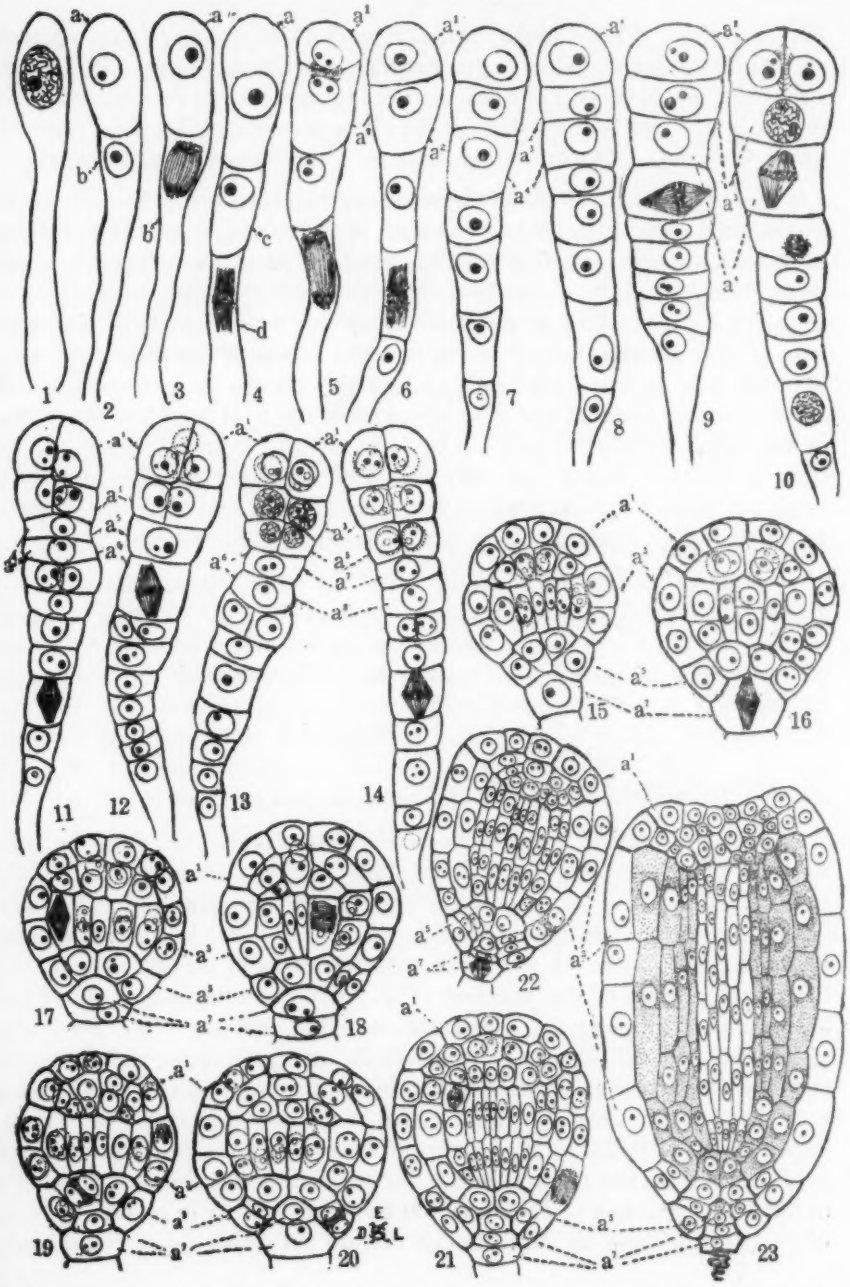
In a previous paper of ours (Kausik and Subramanyam, 1945 *a*) a detailed account of the development of the male and female gametophytes and endosperm formation in *Isotoma longiflora* Presl. has been given. The present paper deals with the development of the embryo in this plant. During this study a single case of polyembryony was met with and this has been separately described (Kausik and Subramanyam, 1946).

Fixation of the material was done in Formalin Acetic Alcohol and the sections were stained in Heidenhain's iron-alum-hæmatoxylin with eosine as counterstain.

The fertilised egg elongates rapidly and becomes tubular with the nucleus situated at the apex (Fig. 1). The first division of the fertilised egg takes place after the endosperm has passed through its initial development. It divides in a transverse manner cutting off a primary embryonal cell (Fig. 2 *a*) and a primary suspensor cell (Fig. 2 *b*). Of these it is the primary suspensor cell (Fig. 2 *b*) that divides first by a transverse wall to form a middle cell *c* and a basal cell *d* (Fig. 4). Thus a three-celled proembryo is formed. In this respect *Isotoma longiflora* resembles *Campanula patula* L. (Souéges, 1936), *Lobelia amœna* (Hewitt, 1939), and *L. trialata* Buch-Ham. (Kausik and Subramanyam, 1945 *b*). In *L. syphilitica* L. (Crete, 1938) and *Cephalostigma Schimperi* Hochst. (Kausik and Subramanyam, in Press), however, it is the primary embryonal cell that divides first. In the three-celled proembryo the middle and basal cells (Figs. 3, 4 and 5) usually divide by further transverse walls, thus adding to the length of the suspensor (Figs. 6 to 14).

The primary embryonal cell *a* now divides first by a transverse wall (Fig. 5) cutting off an apical cell a^1, which does not divide further until longitudinal divisions begin, and a second embryonal cell a^2. The second cell of the proembryo a^2 divides by a transverse wall to form cells a^3 and a^4 (Figs. 7 to 9). At about this stage one of the suspensor cells, usually in the upper region of the filamentous proembryo, divides by a vertical wall (Figs. 9 and 10) to form two cells, which are characteristically seen in the early stages of embryogeny (Figs. 11 to 14). A similar feature is seen in *Jasione montana*



FIGS. 1–23. *Isotoma longiflora* Presl.—Stages in the development of the embryo. For explanation see text. Figs. 1–20. ×1260. Figs. 21–23. ×900 (Original magnifications given here, but figures have been reduced to half in reproduction).

Linn. (Souéges, 1938), *Lobelia amæna* (Hewitt, 1939) and *L. trialata* (Kausik and Subramanyam, 1945 *b*). In *Lobelia syphilitica* (Crete, 1938) and *Cephalostigma Schimperi* (Kausik and Subramanyam, in Press) a group of four cells is formed by the activity of one of the suspensor cells. In *Campanula patula* (Souéges, 1936) more than four cells are formed in this region.

The cells a^1 and a^3 of the filamentous proembryo divide by two sets of longitudinal walls (Figs. 10 to 13) at right angles to each other, thus resulting in two tiers of cells with four cells in each tier. Almost simultaneously with these divisions cell a^4 divides by a transverse wall producing cells a^5 and a^6 (Figs. 10 and 11). Cell a^5 then divides by two vertical walls so that three tiers of cells are now formed in the terminal region of the proembryo (Fig. 14) with four cells in each tier. Cell a^6 also divides by a transverse wall adding two more cells a^7 and a^8 to the terminal region (Figs. 12 to 14). Thus in this region five tiers of cells can be made out *viz.*, a^1, a^3, a^5, a^7 and a^8, the three upper tiers having four cells each and the lower two having only a single cell each. Of these five tiers it is only the first four tiers that actually take part in the formation of the various regions of the embryo.

In the distal tier a^1, anticlinal divisions occur followed by periclinal divisions to form the dermatogen in this region (Fig. 15). The first anticlinal divisions in this tier can be traced in the various stages upto the formation of the mature embryo (Fig. 23). The next division in tier a^2 is tangential cutting off a dermatogen peripherally from a group of inner cells (Fig. 15). The inner cells divide longitudinally separating the future periblem from the plerome. Both longitudinal (Fig. 18) and transverse divisions (Fig. 17) occur in the further development of the plerome and periblem (Figs. 18 to 23).

When the primary body regions are differentiated in the first two tiers, the third tier a^5 develops into a semicircular layer of cells at the base of the embryo. To start with, this layer is made up of four cells (Figs. 15 to 17); but subsequently forms about 8 cells (Figs. 22 and 23) by further oblique divisions (Figs. 18 to 21). The innermost two cells of this group take part in the completion of the periblem (Fig. 23). The remaining cells of this layer help to complete the dermatogen and the root cap. The single cell of tier a^7 does not divide any further until the embryo is rather well developed and spherical in shape. Then it divides by a transverse wall (Fig. 16) forming a proximal and a distal cell (Figs. 17 to 20). Both these cells divide transversely (Figs. 21 to 23) forming a part of the root-cap which is also increased on all sides from tier a^5 and also by extra cells cut off from the dermatogen in tier a^3. According to Hewitt (1939) however, in *Lobelia amoena* the cells of the proximal row, cut off from this tier, do not divide again, but the cells

of the distal row divide transversely separating the periblem from the dermatogen. He further states that the cells of the proximal row become a part of the root-cap. In a mature embryo (Fig. 23) the body regions can easily be assigned to the primary tiers, *viz.*, a^1, a^3, a^5 and a^7, which are clearly recognizable in the early stages of embryogeny. Thus from tier a^1 arise the cotyledons, the stem tip forming in the notch between them; from tier a^3 the hypocotyl is formed with its central row of long and narrow plerome cells, the outer zone of much larger periblem cells (the region shown dotted in Fig. 23) and the outermost layer of dermatogen; from tier a^5 the completion of the periblem and the organisation of a part of the root cap take place; and lastly, from tier a^7 the rest of the root-cap is formed.

Acknowledgment

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THE NEWLY HATCHED LARVA OF *PERICLIMENES (ANCYLOCARIS) BREVICARPALIS* (SCHENKEL)

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(Communicated by Prof. S. G. M. Ramanujam, F.A.SC.)

Introduction

The occurrence of *Periclimenes* (*Ancylocaris*) *brevicarpalis* in association with *Stoichactis giganteum* (Forskal.) at Krusadai Island has been recorded by Gravely (1927) in his study on the littoral Decapod fauna of the Island. Kemp (1922) has collected the species from the giant sea-anemone *Discosoma* at Port Blair. The genus *Periclimenes* comprises of a large number of species about the larval history of which very little is known. Gurney (1936 and 1938) has described the larval stages of the following species of *Periclimenes* (subgenus *Ancylocaris*); *e.g.*:—*calmani*, *americanus*, *agag*, *diversipes* and *grandis*. As the larval history of *Periclimenes* (*Ancylocaris*) *brevicarpalis* is not known it was thought useful to describe the newly hatched larva.

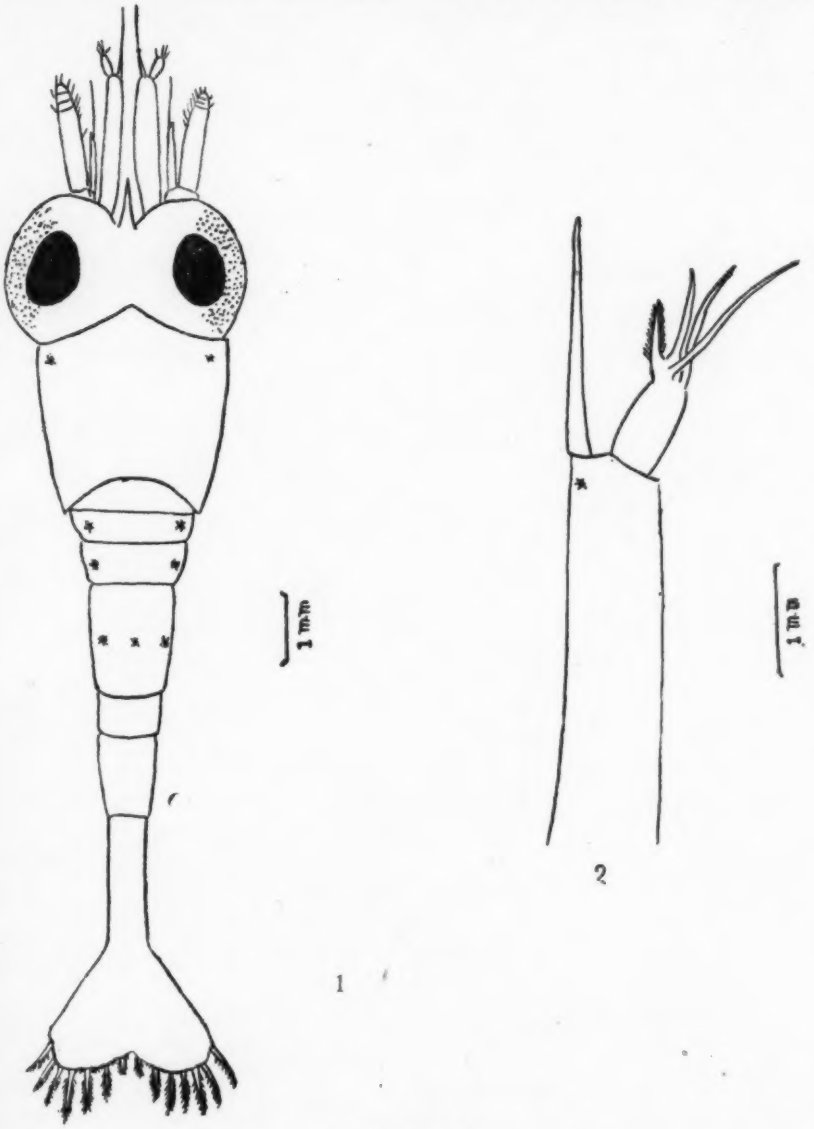
During a brief stay at the Krusadai Biological Station in the Gulf of Manaar, in March 1947, the author collected numerous specimens of *Periclimenes brevicarpalis* including a good number of berried forms from Kundugal Point where *Stoichactis giganteum* occurs in abundance. Generally each anemone shelters a male and a female under the exposed flattened tentacle-bearing region, and even when the anemone contracts they very seldom make an attempt to swim away. Though the prawns are transparent, as observed by Gravely (1927) certain regions of the body are coloured very prominently as described by Kemp (1922).

From a very close observation of a large number of forms, the present author while confirming Gravely's observation is also led to the conclusion that this prominent colouration resembling that of broken shells helps the prawns to escape observation when the anemone contracts and withdraws into its burrow leaving the prawns exposed.

Description of the Newly Hatched Larva

The eggs of some of the berried females on microscopic examination seemed to be in an advanced stage of development, and were therefore kept under observation in the laboratory aquaria. Aeration of the eggs

appears to be effected by the side-to-side rocking movement of the entire animal aided by the gentle movement of the pleopods. The eggs measuring 0·41 mm. to 0·31 mm. in diameter hatched out in the evening. The newly



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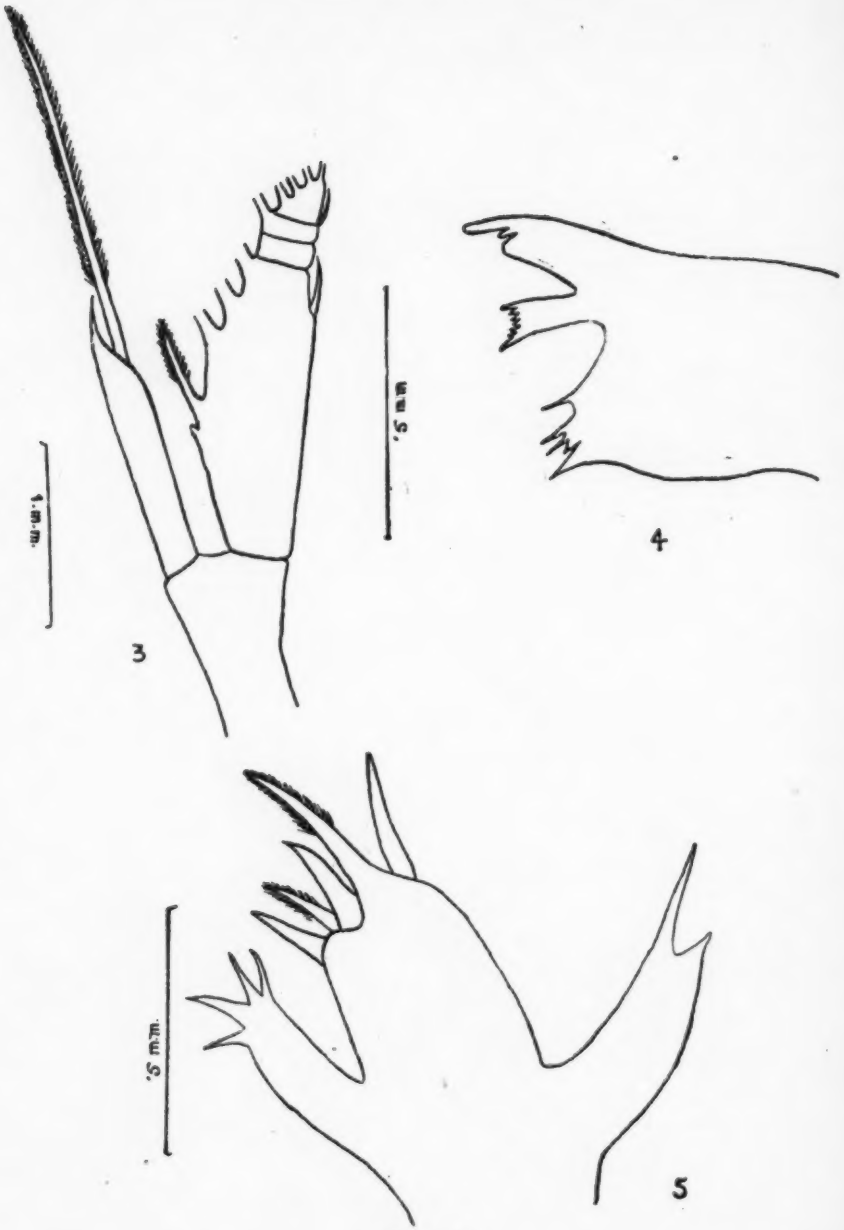
FIGS. 1 & 2



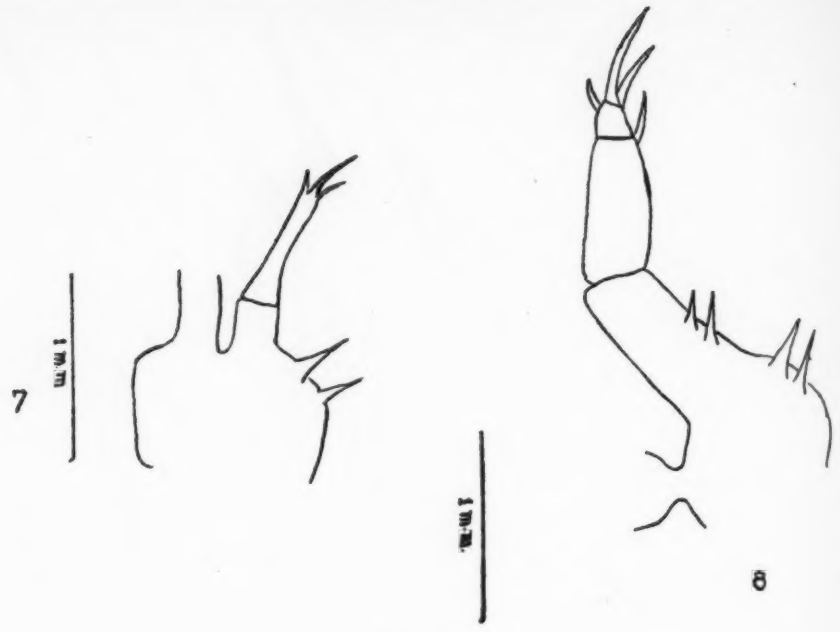
FIGS. 3–5

hatched larvæ, 1·65–1·7 mm. in length (Fig. 1), are very active in their movement and phototactic in behaviour.

Coloration.—The larva is perfectly transparent. Two orange coloured chromatophores are present on the sides of the anterior region of the carapace. On either side of the first abdominal somite is present a shining yellow chromatophore. Chromatophores of the same colour and disposition are present on the second and third abdominal somites, the latter carrying a median dorsal chromatophore in addition. Similar chromatophores are present on the endopods of the second and third maxillipedes.

The eyes.—The eyes are sessile.

Antennule (Fig. 2).—The peduncle is unsegmented. The inner flagellum is in the form of a single large seta. The outer flagellum is represented by a small papilla bearing at its tip three æsthetes and a short plumose seta.



FIGS. 7–8

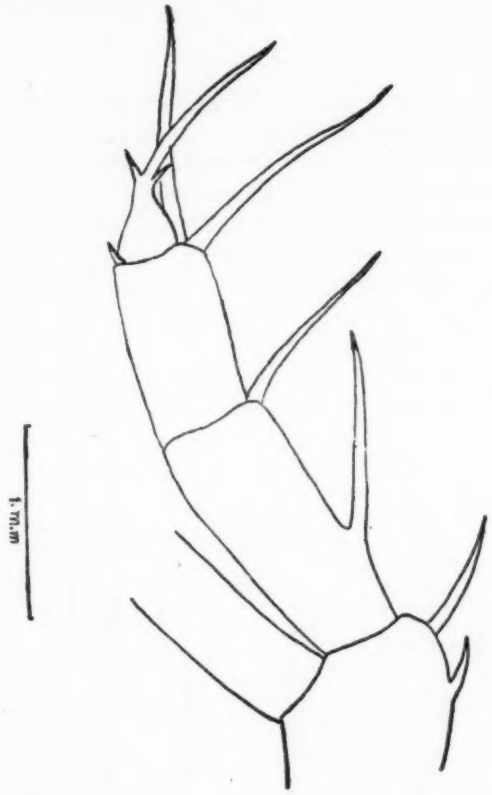
On the peduncle, at the base of the inner flagellum, is a shining golden yellow stellate chromatophore.

Antenna (Fig. 3).—The peduncle is unarmed. The inner flagellum is cylindrical, unsegmented and carries a spine and a long plumose seta. The inner flagellum inclusive of the spine is about two-thirds the length of the scale. The scale is divided into three distinct segments and carries a small papilla and nine plumose setæ along its inner margin. There are two spines on the outer margin, of which the proximal one is longer.

Mandible (Fig. 4).—The mandible is clearly marked off into the incisor and molar regions. The incisor part carries three blunt teeth and the molar part has five pointed spines. In between the incisor and the molar regions is a blade which is serrated at the tip.

Maxillule (Fig. 5).—Endopod of maxillule is bilobed and carries a small seta. The proximal masticatory process is narrow and has four setæ. The distal process is provided with two plumose setæ and three pointed spines.

Maxilla (Fig. 6).—There are three masticatory processes on the protopodite. The proximal process carries two setæ, the middle and the distal



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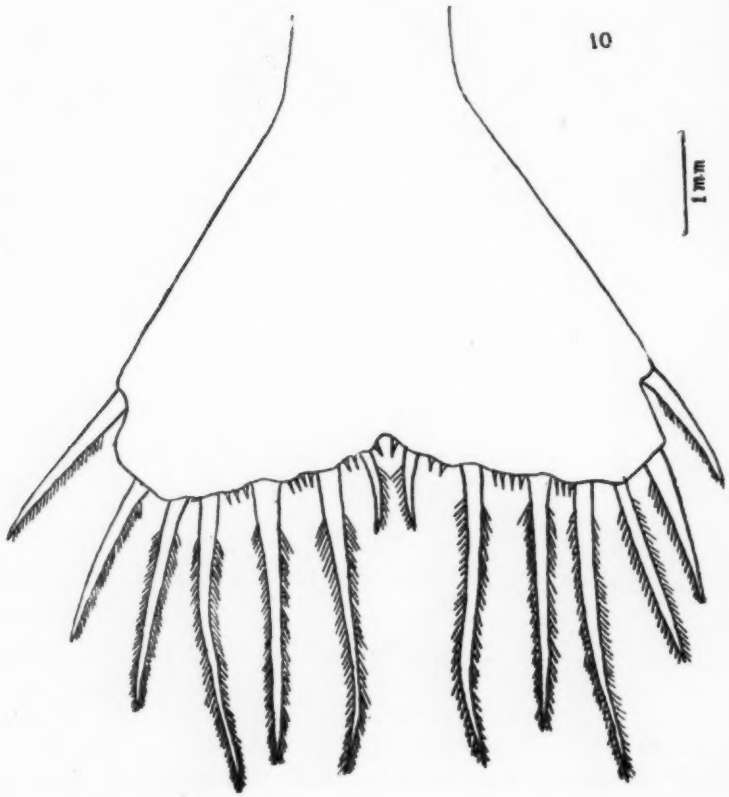
processes bear one and two setæ respectively. The endopodite is not bilobed and is tipped with a single seta. The scale carries four plumose setæ.

Maxillipede 1 (Fig. 7).—Basipodite is not protuberent and carries two spines. Endopodite is bisegmented and carries three terminal spines. The exopodite is unsegmented and carries four setæ.

Maxillipede 2 (Fig. 8).—Coxopodite is unarmed. Basipodite has two spines. The endopod is distinctly three segmented. The basal segment on its inner side carries two spines. Another spine is placed at the base of the third segment. The third segment carries two long terminal setæ and one small spine on its outer margin. Exopodite is unsegmented and is similar to that of the previous appendage.



Maxillipede 3 (Fig. 9).—Basipodite carries two spines. The endopodite is of three distinct segments of which the first and second bear on their inner margin one spine each. At the base of the second segment on its inner margin is another spine. Dactyl ends in a single strong terminal spine and carries two small spines, one on its outer side and another on its inner side. At the base of the terminal segment on its inner side is a small spine.

Telson (Fig. 10).—The telson is slightly concave on the ventral side. The usual seven pairs of spines are present. The fourth spine is slightly

longer than the sixth. The innermost is the smallest and is enclosed in a sheath in the embryonic condition.

Remarks

Gurney (1938) has summarised the larval characters of the genus *Periclimenes* (*Ancylocaris*), based on the larval stages of *grandis*, *agag*, *calmani*, *diversipes* and *americanus*. Gurney (1938) has also emphasised the fact that the larval characters mentioned are applicable in general to the larvæ of the genus *Periclimenes* exception being made to *Periclimenes* (*Ancylocaris*) *diversipes*. The larva of *Periclimenes* (*Ancylocaris*) *brevicarpalis* shows some interesting features in comparison to the other species.

Name of Species	Appendages			
	Maxillule	Maxilla	Maxillipede I	Maxillipede III
P (A) agag	endopod bilobed	endopod has a basal lobe. Scale has four plumose setæ	endopod not distinctly segmented. Basis protuberant	Dactyl ends in two strong terminal spines
P (A)diversipes	endopod not bilobed	endopod has no basal lobe. Scale has five plumose setæ	endopod unsegmented, basis slightly protuberant	Dactyl ends with a single strong terminal spine
P (A) grandis	endopod bilobed	Basal lobe present in the endopodite. Scale has four plumose setæ	endopod not distinctly segmented	Dactyl ends in two long subequal terminal spines
P (A) brevicarpalis	endopod bilobed	Basal lobe of endopod absent. Scale has four plumose setæ	Basipodite not protuberent. Endopodite bisegmented	Ends in a single strong terminal spine

It was not possible to study the subsequent stages owing to the shortness of the author's stay at the station, nevertheless these few observations are recorded here in the hope that the opportunity for continuing the work will be available in the near future.

Acknowledgement

I wish to express my gratitude to Dr. C. P. Gnanamuthu, M.A., D.Sc., F.Z.S., Director, University Zoology Laboratory, Madras, for his help and criticisms throughout the course of this work.

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EXPLANATION OF FIGURES

FIGS. 1–10. The newly hatched larva of *Periclimenes* (*Ancylocaris*) *brevicarpalis* and its appendages.—Fig. 1. The newly hatched larva. Fig. 2. Antennule. Fig. 3. Antenna. Fig. 4. Mandible. Fig. 5. Maxillule. Fig. 6. Maxilla. Fig. 7. Maxillipede 1. Fig. 8., Maxillipede 2. Fig. 9. Maxillipede 3. Fig. 10. Telson.

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ERRATA

Vol. XXVI, No. 2, August 1947, Sec. B.

On page 69, third line from bottom, "0·028 gm. of nitrogen" *should read* as "0·28 gm. of nitrogen".

The caption of the Y-axis of text-figures 2 and 4 on pages 70 and 72 should read as *macroconidia* and not *microconidia*.

ERRATUM

Vol. XXVI, No. 2, August 1947, Sec. B

Plate IV:—read 1, 4 and 7 in place of 3, 6 and 9; and 3, 6 and 9, in place of 1, 4 and 7.

COPEPODS OF THE WEST HILL SEA*

By P. K. Jacob and M. Devidas Menon

Received April 2, 1947
(Communicated by Prof. Beni Charan Mahendra, D.Sc., F.Z.S., F.A.Sc.)

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I. Introduction

The importance of Copepods in plankton cannot be over-emphasized. As pointed out by Johnstone Scott and Chadwick (1924), they " are ubiquitous

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and abundant and of prime economic importance, in the respect that they are plentiful source of food both for many pelagic animals like Herring and for hosts of small fishes". They constitute a major group of organisms in the zoo-plankton, contributing (as found by us) as much as 75 to 90 per cent of the total population of the planktonic organisms in the West Hill area of the Arabian Sea and forming " one of the essential links in the food chain of the sea " (Clarke, 1939). They occur almost throughout the year in this area also, just as in the sea off the Trivandrum Coast (Menon, 1945) and in the Madras Coastal waters (K. S. Menon, 1931).

Although Copepods occur throughout the year in the West Hill plankton, they undergo seasonal fluctuations in number, owing to the varying population of diatoms, which depends upon the hydrographic and meteorological conditions. The present communication is an attempt to trace these relationships for the five years, beginning from July 1939 and ending with June 1944.[1] A list of the genera and species of Copepods abounding in the West Hill area is recorded, and the seasonal fluctuations of six important genera of Copepods have been traced. For one year (1945–46) the fluctuation has been studied in greater detail with special reference to its correlation to diatoms and physical factors. In order to add to our knowledge of the food chain of the sea, the authors have tried to correlate the Copepodan abundance with the amount of plankton-feeding fishes in general and the Indian Chub Mackerel (*Rastrelliger kanagurta*) in particular, landed on the Calicut Coast.

II. Physical Features and Climatic and Hydrographical Conditions of the Coast

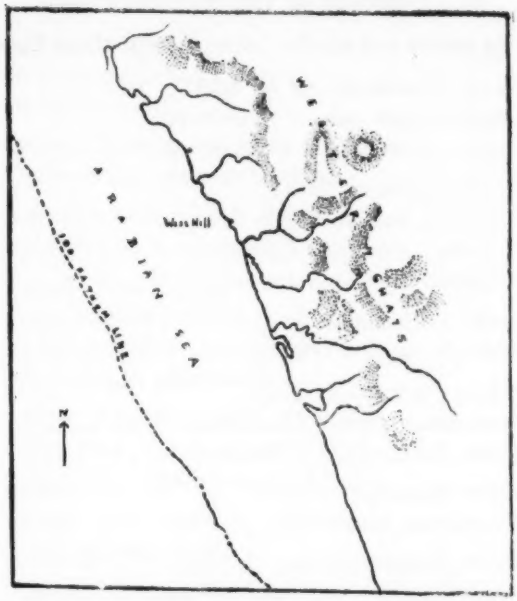
(*a*) *Physical Features*

The West Coast of Madras is characterised by a narrow strip of low-lying land between the high mountains of Western Ghats, rising to an average height of 5,000 feet, and the Arabian Sea (Fig. 1). Naturally, the rivers flowing into the sea are very short. The descent from the mountains being steep, these rivers flow in great torrents, carrying rich debris into the sea. Perhaps this feature is responsible for the wide Continental Shelf fifty miles broad here (Raj, 1931).

(*b*) *Climatic Conditions*

Rainfall.—The West Coast of Madras receives rain from two monsoons. The greater part is obtained from the South-West Monsoon which sets in

[1] The computation is in accordance with the Fisheries year, beginning in July of one calendar year and ending in June of the following year.



TEXT-FIG. 1. *Map of the Malabar Coast.* (Scale 1 inch = 28 miles)

May or June and ends in August. During these months, the sea is very turbulent and turbid. The North-East Monsoon occurs in October and November (Fig. 4). The average rainfall at West Hill is 107·5".

(*c*) *Hydrographical Conditions*

Specific Gravity.—The lowest specific gravity is in the months of June, July and August. In all the other months except in November, in which month there is a slight fall, the specific gravity curve maintains an average high level (Fig. 4) (Chidambaram and Menon, 1945).

Surface Temperature.—According to Sewell (1925–29), the surface temperature of Indian seas directly depends upon the monsoon. Our investigations confirm this statement. The lowest surface temperature is noticed to occur in the months of June to September, *i.e.*, during the South-West Monsoon. The highest peak is reached in the months of March, April and May. The little fall in the surface temperature in the months of December and January is due to the North-East Monsoon and the currents (Fig 4) (Chidambaram and Menon, 1945).

III. Copepods of the West Hill Area

The following genera and species form the bulk of the Copepod group:—

Genus *Canthocalanus* A. Scott.

1. *Canthocalanus pauper* (Giesbrecht).
2. *Calanus finmarchicus* (Gunnerus).

Genus *Eucalanus* Dana.

3. *Eucalanus crassus* Giesbrecht.
4. *Eucalanus suberasus* Giesbrecht.
5. *Eucalanus attenuatus* Dana.

Genus *Undinula* A. Scott.

6. *Undinula vulgaris* (Dana).

Genus *Paracalanus* Boeck.

7. *Paracalanus parvus* Giesbrecht.
8. Genus *Pseudocalanus* Boeck.

Genus *Rhincalanus* Dana.

9. *Rhincalanus nastus* Sars.

Genus *Pseudodiaptomus* Herrick.

10. *Pseudodiaptomus annandalei* Sewell.

Genus *Temora* W. Baird.

11. *Temora discaudata* Giesbrecht.
12. *Temora longicornis* (Muller).

Genus *Centropagus* Kroyer.

12. *Centropagus furcatus* Dana.
13. *Centropagus tenuiremis* Thompson and Scott.

Genus *Labidocera* Lubbock.

14. *Labidocera acuta* (Dana).

Genus *Metridia* Boeck.

15. *Metridia lucens* Boeck.

Genus *Candacia* Dana.

16. *Canadacia truncata* Brady.

Genus *Pontella* Dana.

17. *Pontella danæ* Giesbrecht.
18. *Pontella securifer* Brady.

Genus *Acartia* Dana.

19. *Acartia erythrea* Giesbrecht.
20. *Acartia discaudata* Giesbrecht.

Genus *Oithona* Baird.
21. *Oithona rigida* Giesbrecht.
22. *Oithona plumifera* Baird.

Genus *Clytemnestra* Dana.
23. *Clytemnestra rostrata* (Brady).

Genus *Microsetella* A. Scott.
24. *Microsetella rosea* Dana.

Genus *Oncæ* Phillipi.
25. *Oncæ venusta* Phillipi.
26. *Oncæ confifera* Giesbrecht.
27. *Oncæ ornata* Giesbrecht.

Genus *Sapphirina* J. W. Thomson.
28. *Sapphirina ovatolanceolata* Dana.
29. *Sapphirina nigromaculata* Claus.

Genus *Isias* Boeck.
30. *Isias clavipes* Boeck.

Genus *Corycæus* Dana.
31. *Corycæus elongatus* Claus.
32. *Corycæus venustus* Dana.
33. *Corycæus furcifer* Claus.

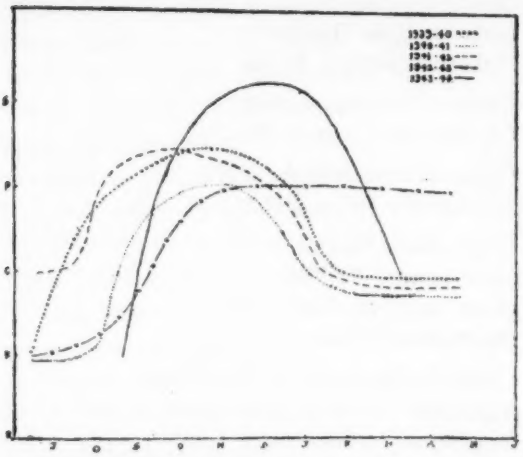
Genus *Euterpina* Norman.
34. *Euterpina acutifrons* (Dana).

IV. Fluctuations of Copepods in General in the West Hill Sea for the Quinquennium 1939–40 to 1943–44

(Text-Fig. 2)

1939–40.—In July 1939, the Copepods occurred scarcely, but in August and September there was a steady rise, so that the 'Plenty'[1] stage was reached in September and even exceeded in October. However, it was during November that the maximum was reached, which was half way between the 'Plenty' and the 'Swarm' stages. From December onwards there was a steady fall, a reversal of the rise noticed in August and September. In January the level fell to the 'Common' stage and from February onwards a slightly lower level than this was maintained. Comparing the Copepodan abundance of this year with those of the other four years,

[1] In the rough quantitative estimation of planktonic organisms made in the West Hill Biological Station, J. Hornell applied the following terms in the increasing order 'Rare', 'Few', 'Common', 'Plenty' and 'Swarm'. This quantitative analysis is being still followed.



TEXT-FIG. 2. Chart showing the Annual Fluctuations of Copepods of the West Hill Sea for the quinquennium 1939 to 1944. Months are shown on the horizontal axis and the frequency of copepods on the vertical. *J*, *A*, *S*, *O*, *N*, *D*, *J*, *F*, *M*, *A*, *M*. *J*, = months from July to June. *R* = Rare. *F* = Few. *C* = Common. *P* = Plenty. *S* = Swarm.

the Copepods seem to have maintained a fairly high level for more than an average length of time, which phenomenon may be attributed to the normal climatic and hydrographical features of this year favouring the abundance of diatoms (*i.e.*, the food for Copepods) present in the sea, especially at the beginning of the year.

1940–41.—This year was not a favourable one like the previous one for Copepods. Almost up to the month of September, the Copepods were 'Few' in plankton. It was during this month that the quantity of Copepods increased towards the 'Plenty' stage, but it never exceeded this stage. The decline began earlier this year, *i.e.* in December, and by January the level fell below the 'Common' stage from which it never rose again. On the whole, this was a very poor year for Copepods, probably due to such adverse hydrographical conditions as high temperature, specific gravity, etc. (Chidambaram and Menon, 1945).

1941–42.—During this year, conditions seem to have been quite fair for Copepods. This year's fluctuation simulates that of 1939–40 with the minor difference that the Copepods flourished better until the month of October and were less abundant afterwards. The fair weather with a large number of sunny days seems to have had a beneficent effect on the copepodan abundance.

1942–43.—The fluctuation curve of Copepods takes a different aspect this year from that of the other years under review. The rise to the ' Plenty ' stage is reached rather late in November, but once it has risen to this stage it is maintained uniformly throughout the rest of the year. The unusually prolonged monsoon and the very low surface temperature of this year account for this peculiar curve (Chidambaram and Menon, 1945).

1943–44.—The late appearance of Copepods in the sea and the unprecedented rise in their abundance characterize this year. The ' Swarm ' stage was maintained in December and January. In February the fall was as steep as was the rise in September. The diatom abundance alone seems to have been responsible for the swarming of Copepods this year.

The fluctuation curves for the three years 1939–40, 1940–41 and 1941–42 resemble each other in showing a rise in abundance during the months of July and August, a high level having been reached in all cases in September and maintained during October, November and December, this being followed by a low level in January. The differences from such a frequency curve noted in the years 1942–43 and 1943–44 are accounted for by climatic changes and variations in the abundance of diatoms which form the food of Copepods.

Regarding the plankton of the Malabar coast, Hornell and Ramaswamy Naidu (1923) reported that Copepods are the dominant crustaceans met with in the plankton. They first appear in quantity in November, increasing to their maximum abundance early in December but suffering no appreciable diminution till January. In March they are reduced to five-eighths, and they are very scarce from April to August.

The course of Copepodan fluctuation noticed in the present investigation differs from that observed by Hornell and Naidu. According to them, " they appear in quantity in November ", whereas a fairly high level was noticed by us to have been reached much earlier (in September) in almost all the years under review. Their statement that in January the number of Copepods are not reduced does not agree with our observations, since in all the first three years the Copepods fell down in number and reached low levels in January.

In 1942 Devanesan and Chidambaram stated that " the entomostracan Copepoda should lead any list of planktonic organisms made in the sea opposite West Hill, for such is its abundance and constancy in its occurrence that it mostly occupies the ' Swarm ' stage and falls only to ' Plenty ' stage". However, they noted falls in the months of August in 1936–37 and July in 1940–41.

The fluctuation curves of Copepods of the West Hill plankton for the five years under review (Fig. 2) shows that the Copepods as a group are found to be at their lowest ebb from May to September. From this month onwards there is a greater Copepodan activity and the population of Copepods in the plankton increases so that they reach their maxima in the last week of November and early week of December. The fluctuation of Copepods shows unimodal curves indicating a Copepodan abundance for a prolonged period from September to January reaching peaks in December when they assume the 'Swarm' stage in the plankton. From January onwards they begin to decline.

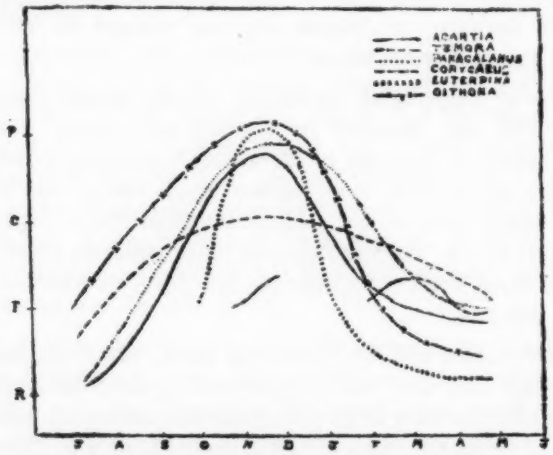
V. Seasonal Fluctuations of Six Important Genera of Copepods of the West Hill Sea for the Year 1945–46

As in the Madras plankton, two types of Copepods were recognized in the West Hill plankton according to the nature of fluctuation and appearance that they exhibited:

(1) Those which closely follow the total Copepodan fluctuation, like *Oithona*, *Paracalanus*, etc.

(2) Those which exhibit short sharp maxima, but are almost altogether absent at other times, like *Euterpina* and *Corycæus*.

This was established from a study (Fig. 3) of the appearance and fluctuations of the following six genera:—



Text-Fig. 3. Chart showing the Seasonal Fluctuations of six Important Genera of Copepods of the West Hill Sea, for the year 1945–46. (For explanation of abbreviations, see Fig. 2.)

Oithona, Paracalanus, Acartia, Temora, Euterpina and *Corycæus*

Oithona.—This is the first Copepod to appear in the plankton in the month of July together with some Crustacean and Copepodan larvæ and post-larval stages. This form is found throughout the year in the plankton and has its maximum period in November and December.

Paracalanus.—*Paracalanus* is the most common form in the plankton of this coast. It is very rare in July, but becomes common gradually, reaching its period of maximum abundance in the latter part of October; it continues to be fairly abundant till the end of March. The peak period is from November to January.

Acartia.—*Acartia* is continuously present in the plankton and its fluctuation is very similar to that of *Paracalanus*. The peak period, however, is slightly shorter than that of *Paracalanus*.

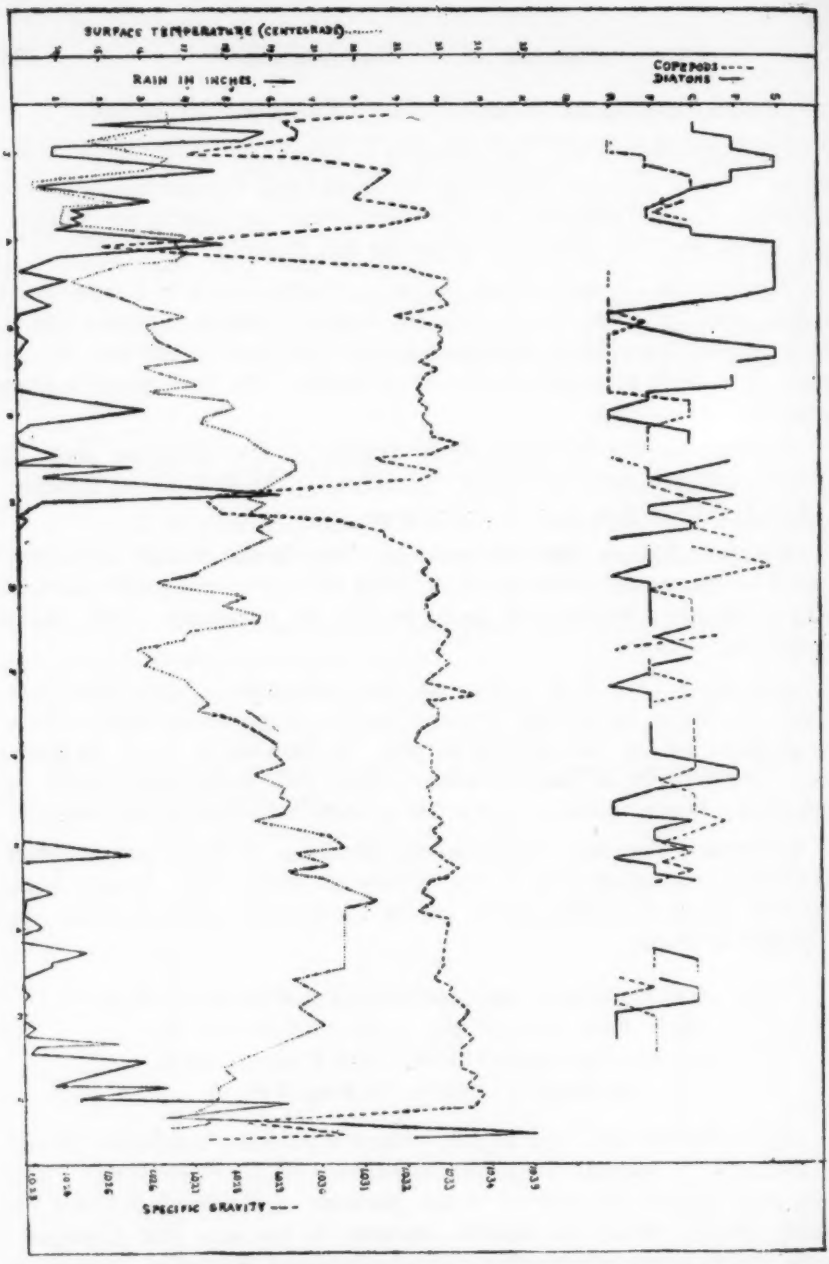
Temora.—*Temora*, like the previous three forms, is also constantly present in the general plankton of the West Hill area; but unlike them, it lacks a sharply distinguished peak period, its fluctuation curve being smooth and uniform.

Euterpina.—This form makes its first appearance much later than others, in August in the first instance, and it disappears completely from the plankton by the last week of August. In October, it comes up again and is followed by a sharp maximum which falls down considerably in December. Hence onwards only a few of them are found in the plankton.

Corycæus.—*Corycæus* occur in the plankton in those months when the surface temperature and specific gravity are fairly high. During June, July and August when the specific gravity and surface temperature are low *Corycæcus* is absent.

VI. Fluctuation in the Copepod Population of the West Hill Area within the year 1945–46 and its Correlation with Diatoms and Hydrographic and Meteorological Factors (Fig. 4)

July.—The first ten days in July present a plankton completely devoid of Copepods. Copepods make their appearance on the eleventh day. The same phenomenon was noticed in the plankton of the Madras Coast by Menon (1931). When the diatoms increase, it was seen that Copepods closely follow them (Chidambaram and Menon, 1946). The production of Copepods halts during the period when the diatoms reach the stage of ' Swarms ', as was noted in the plankton of the Trivandrum Coast.



TEXT–FIG. 4. Chart showing the fluctuation in the Copepod population of the West Hill area within the year 1945–46 and its correlation with Diatoms and hydrographic and meteorological factors. The upper curves represent the frequency of Copepods and diatoms; when the latter three curves show the variations in surface temperature, rainfall and specific gravity during the various months of the year 1945–46.

From the third week Copepods tend to increase in numbers as well as in species and with this comes the fall in diatoms. During the last days of the month, both Copepods and Diatoms begin to wane down and assume more or less the same intensity in population.

August.—The condition at the beginning of the month shows still a downward trend. A slight increase in temperature and salinity and bright sunshine set in and a sudden outburst of diatoms follow. The Copepods follow the upward trend to some extent. Towards the third week heavy turbulence of the sea with a strong current attended with bright sunshine and slight rise in temperature, cause the phenomenon of " Animal exclusion " from the plankton. These conditions are most favourable for the creation of a diatom maxima and " zoo-plankton definitely avoids area where phyto-plankton is thick" (Hardy, 1935). This diatom flowering is continued to the end of the month and is carried to the beginning of the next month.

September.—The diatom flowering soon falls for a short period during which favourable conditions prevail for a slight increase in the activity of the Copepods. Again, simultaneous with the recurrence of fairly identical conditions as in August there is a repetition of the descent of the Copepod curve and an ascent of the diatom curve.

October.—The Copepods are at their lowest ebb at the beginning of the month and consist of only a few specimens of *Acartia*, while the diatoms abound. Thenceforth, the variety and quantity of Copepods increase and *Temora*, *Paracalanus*, *Euterpina* and *Pseudocalanus* come into the planktonic picture. This condition together with the scarcity of diatoms continue for a short period, when due to the scarcity of diatoms the Copepods fall down.

November.—From the beginning of November, there is a gradual ascent of the Copepoda with great enhanced activity of *Paracalanus*, *Acartia*, *Euterpina*, *Oithona* and *Pseudodiaptomus*. Diatoms show a weathering out and are very rare towards the end of the month.

December.—At the beginning of December the Copepods are at their zenith of activity. The abundance of Copepods in December is similar to the peak of diatoms in August and September. December is a zoo-planktonic month.

January and February.—In January there is an initial fall of Copepods which is soon made up and throughout the rest of the month and in February excepting the last week there is a steady production of Copepods.

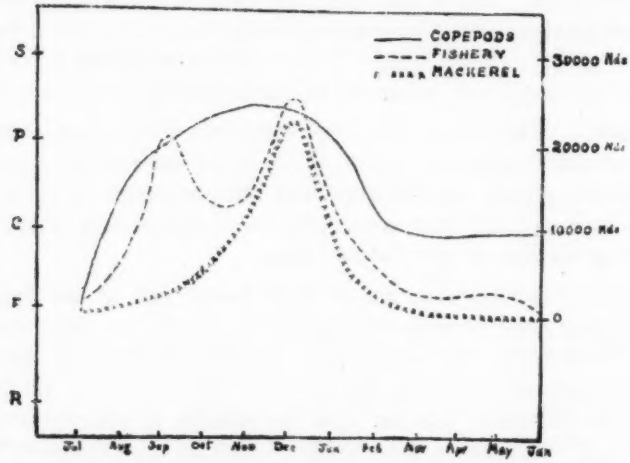
March, April, May and June.—From March onwards there is only a very reduced activity of Copepods and they descend to very ' Rare ' stage in May and June.

VII. Copepod Fluctuation in Relation to West Coast Fishery for the Quinquennium 1939–40 to 1943–44

The fluctuations in the Copepod population in the West Hill plankton were correlated to those of the fishery of the plankton-feeding fishes of the Calicut Coast for the years 1939–40, 1940–41, 1941–42, 1942–43 and 1943–44.

1939–1940 (Fig. 5)

The Copepods begin to rise to a high level by September and continue to be in the peak period till December. The general fishery of the Coast

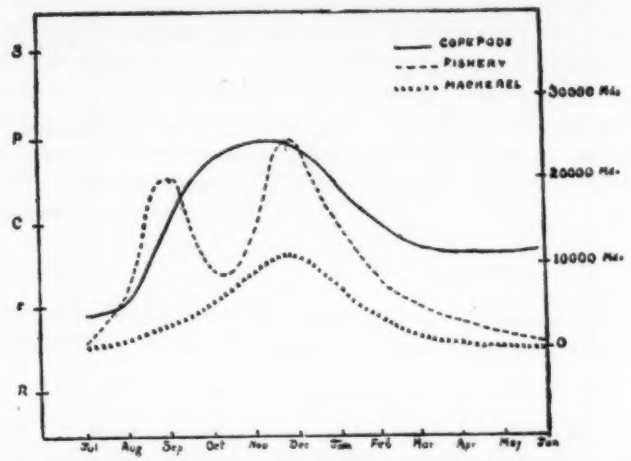


Text-Fig. 5. Chart showing the correlation of Copepods with general fishery and Mackerel Fishery of the West Hill Sea for the year 1939–40.

also flourishes and reaches a peak in September, but falls down to a lower level in October, due to the prevailing North-East Monsoon, during which fishermen find it difficult to go out into the sea. Notwithstanding the temporary fall, the general fishery again rises to its maximum by December, from which month there is a steady fall, simulating the decline of the Copepod curve. As for the Mackerel fishery, the peak in September is absent but that in December coincides with both the general fishery and the Copepod peaks.

1940–1941 (Fig. 6)

The graph differs but slightly from the one just described (1939–40). The Copepods take longer time to establish their abundance and attain

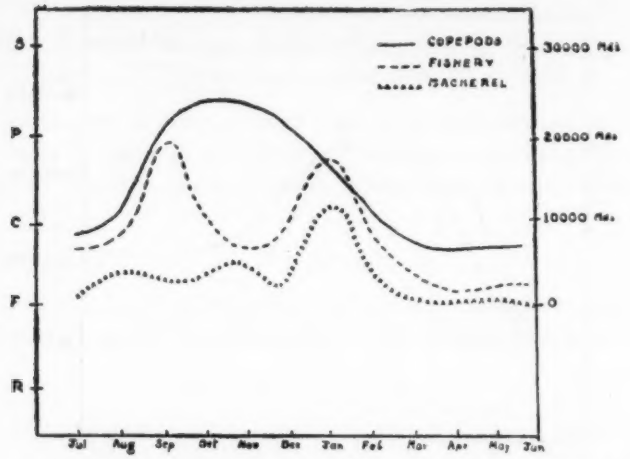


TEXT-FIG. 6. Chart showing the correlation of Copepods with general fishery and Mackerel Fishery of the West Hill Sea for the year 1940–41.

'Plenty' stage in October and maintain that level till January. The general fishery, however, rises to a peak in September, a little ahead of the Copepodan abundance. The fall in general fishery, due to the North-East Monsoon, is repeated this year as well. Nevertheless, the general fishery soon regains its abundance and the highest peak is reached in December, coinciding with the Copepodan maximum. The Mackerel fishery has only one peak as in the previous year, which peak coincides with those of the Copepod and the general fishery. After December, all three, *i.e.*, Copepod, general fishery and Mackerel Fishery, are on the decline.

1941–1942 (Fig. 7)

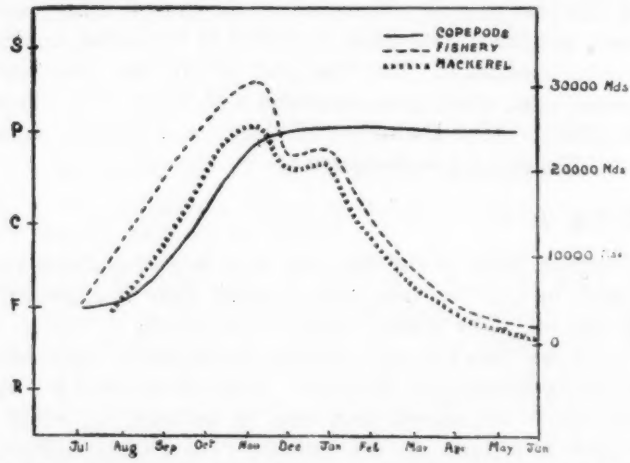
The Copepods begin to rise this year from August onwards and reach a very high peak by October, from which month there is a gradual decrease in their abundance. The general fishery, too, closely following the Copepodan curve, at first rises but later suffers a depression in September, perhaps due to the strong North-East Monsoon. The fishery takes a long time to recover and attains the second peak only in January, by which time the Copepod curve is already on the descent. The Mackerel Fishery, even though it exhibits slight activity in August and again in October, reaches its maximum peak in January, coinciding with those of Copepodan and the general fishery.



TEXT-FIG. 7. Chart showing the correlation of Copepods with general fishery and Mackerel Fishery of the West Hill Sea for the year 1941–42.

1942–1943 (Fig. 8)

The Copepods take a longer time to rise up to the ' Plenty ' stage, this being achieved only in December. From this month, the abundance of



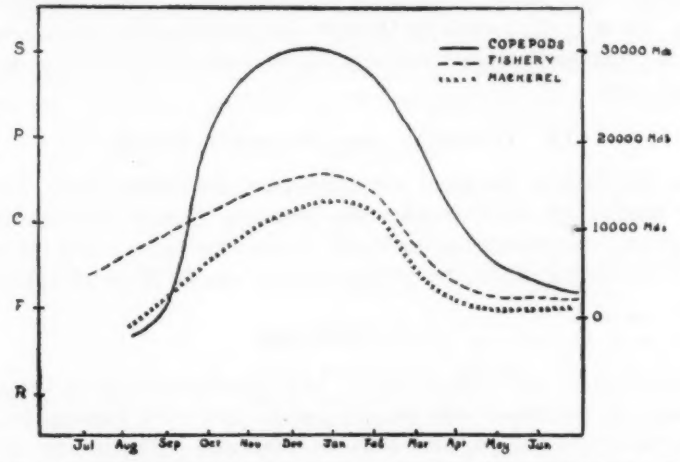
TEXT-FIG. 8. Chart showing the correlation of Copepods with general fishery and Mackerel Fishery of the West Hill Sea for the year 1942–43.

Copepods is maintained at almost the same level for the rest of the year. The general fishery curve runs almost parallel to the Copepod ascent and

the peaks in both are reached simultaneously, but from November onwards there is a slight fall. From this depression, it seems as if the general fishery tried to emerge again, but soon after a slight rise descended to low levels, unlike the steady Copepod curve. The Mackerel fishery curve runs almost parallel to the general fishery curve. The North-East Monsoon being less prolonged does not seem to have had any adverse effect on the fishery. It is not known why the fishery did not maintain a high level following the steady Copepodan abundance.

1943–1944 (Fig. 9)

In September, the Copepods increase in quantity and reach the ' Swarm ' stage in December. From February onwards there is a regular descent



TEXT-FIG. 9. Chart showing the correlation of Copepods with general fishery and Mackerel Fishery of the West Hill Sea for the year 1943–44.

of the Copepod curve. The general fishery is fair from the beginning of the year and its rise is gradual until the zenith is reached in January. From February onwards, there is a descent similar to the Copepod curve. The Mackerel fishery differs but little from the general fishery, the only noticeable difference being its low start at the beginning of the year. The Mackerel fishery also reaches its maximum in January and shows a decline from February onwards, corresponding to the general fishery and Copepods. The absence of a depression in the fisheries during the North East Monsoon was due to the comparatively weak Monsoon which prevailed this year.

VIII. Copepods and Fisheries

During the months of July and August, due to the Monsoon, there is very little fishery and the Copepods too are at their ebb. By September, the Copepods are on the ascent and the plankton-feeders too increase. Due to the North-East Monsoon, there is a fall in the fishery in the months of October and November. It is remarkable that the peak periods for both the Copepods and the Fishery are reached in December for all the five years. This condition extends to January also for two years, *i.e.*, 1942–43 and 1943–44 (Figs. 7 and 8). From February onwards there is a sudden fall in both the fishery and Copepods, probably due to upwelling of the coastal waters. From March onwards up to June, there is neither good fishery, nor do the Copepods rise above the ' Common ' stage. In the year 1942–43, there was a remarkable abundance of Copepods. They ascended to the ' Plenty ' stage in the month of October and continued in that stage for the rest of the year, and naturally the best fishery in all the five years under review occurred then.

IX. Copepods and Mackerel Fishery

The Mackerel is the most important plankton-feeding fish. It is noteworthy that in all the five years the Mackerel fishery presents unimodal curves closely resembling the unimodal copepodan curves, with the peaks of the two coinciding while the general fishery shows bimodal curves (Figs. 4 to 8).

X. Discussion

Chidambaram and Menon (1946) have described in detail the seasonal occurrence of diatoms in the sea off Calicut and their relationship to the physical factors in the sea and weather conditions. The present investigations not only confirm their observations, but tracing the food-chain of the sea further show how the Copepods link diatoms to the fishery. The physical factors have little direct effect on Copepods but they influence the fluctuations of diatoms, which, in their turn cause Copepodal fluctuations. Hence it is that the two monsoons with their freshets of nutrient salts, the slight increase in temperature and salinity, increase in sunlight and calm waters, all seem to have a beneficent effect, though not immediately, on the Copepodal growth.

The correlation between diatoms and Copepods in the plankton is obvious. Earlier workers (Johnstone, 1911; Fish, 1925; Bigelow, 1926) stated that the " main copepodan population appears in times and places where prominent diatom flowerings were absent". But the Monsoons

caused a diatom bloom setting " a nursery for young Copepods " (Wimpenny, 1926). However, these developing Copepoda grazed on the diatom patches " eating holes into it ". So Copepods can be considered as a major factor in regulating the diatom population (Harvey, Cooper, Lebour and Russel, 1935).

The relationship between Copepodal fluctuation and the fishery is also apparent. The fishery of plankton-feeding fishes in general and Mackerel in particular, coincides with the Copepodal fluctuations.

XI. Acknowledgements

This report has been compiled with the help of observations recorded at the West Hill Biological Station for the past one decade. We wish to express our indebtedness to the various research workers who recorded this data, as well as to Professor Beni Charan Mahendra for his constructive criticism and valuable suggestions in the preparation of the manuscript.

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APPENDIX

Fishes of the West Hill Area, found to feed on Copepods

Family *Clupidæ*	..	*Sardinella fimbriata* (Cuv. and Val.).
		Sardinella longiceps (Cuv. and Val.).
		Sardinella albella (Val.).
		Sardinella jussieu (Lee).
		Macrura kelee (Cuv.).
		Macrura ilisha (Buch. and Ham.).
		Opisthopterus tardoore (Swainson).
		Kowala coval (Cuv.).
Family *Engrulidæ*	..	*Thrissocles mystax* (Schn.).
		Thrissocles dussumieri (Val.).
		Thrissocles malabaricus (Bloch.).
		Thrissocles kammalensis (Blkr.).
		Anchoviella tri (Blkr.).
		Anchoviella heteroloba (Ruppel).
		Anchoviella zollingeri (Blkr.).
Family *Dorosonidæ*	..	*Anadontostoma chacunda* (Buch. and Ham.).
Family *Chanidæ*	..	*Chanos chanos* (Forsk.).
Family *Aridæ*	..	*Arius dussumieri* (Cuv. and Val.).
Family *Hemiramphidæ*	..	*Hemiramphus georgii* (Cuv. and Val.).
Family *Cynoglossidæ*	..	*Cynoglossus semifasciatus* (Day).
		Cynoglossus brachyrhynchus (Blkr.).
Family *Mugilidæ*	..	*Mugil parsia* (Buch.).
	..	*Mugil waigiensis* (Q.G.).
Family *Sciænidæ*	..	*Johnius carutta* (Block.).
Family *Scombridæ*	..	*Rastrelliger kanagurta* (Rupp.).
Family *Carangidæ*	..	*Caranx crumenopthalmus* (Blrk.).
		Caranx kurra (Cuv. and Val.).
		Caranx kalla (Cuv. and Val.).
Family *Serranidæ*	..	*Serranus fasciatus* (Forsk.).
Famiy *Polinemidæ*	..	*Polynemus sextarius* (Blkr.).
Family *Lactaridiæ*	..	*Lactarius lactarius* (Schn.).
Family *Leognathidæ*	..	*Leognathus bindus* (Cuv. and Val.).
		Leognathus splendens (Blkr.).
		Leognathus insidiator (Blkr.).
		Leognathus ruconius (Ham.).
		Leognathus edentulus (Blkr.).

SOME STAGES IN THE DEVELOPMENT OF THE PINEAL COMPLEX OF *CALOTES VERSICOLOR* (DAUD.)

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(Communicated by Prof. M. A. Moghe, F.A.Sc.)

Introduction

Since Leydig's[1] pioneer paper dealing with the Parietal Organ in *Lacerta* and in *Anguis* a large number of papers on the structure and development of this organ in a series of vertebrates have been published by various authors. Baldwin Spencer (1887) in his excellent monograph on the " presence and structure of the Pineal eye in Lacertilia " gives a brief description of the structure of Pineal eye in some species of *Calotes*. It seems there is no other work dealing with the Pineal organ of *Calotes* although Dendy (1899 *a*), (1899 *b*), (1907), (1911), Nowikoff (1910), Boveri (1925), and others have described the structure and development of pineal organ in many reptiles. Gladstone and Wakeley (1940) give a summary of all the work done on this organ from morphological, histological, cytological, embryological and medical points of view.

The present paper attempts to give a brief description of some developmental stages of pineal organ in a series of *Calotes* embryos. This study was undertaken while examining a series of sections of the embryos of *Calotes versicolor* (Daud.) for the purpose of observing the development of some of the chondrocranial elements. Unfortunately, very young stages of embryos were not present in my collection.

My study confirms, except in some details, the observations made by previous workers. Whereas the general plan of the development of the pineal organ in *Calotes* in no way differs from that of *Sphenodon* and *Lacerta*, the structure of the Paraphysis in this case is simpler and the lens arises at a comparatively later stage.

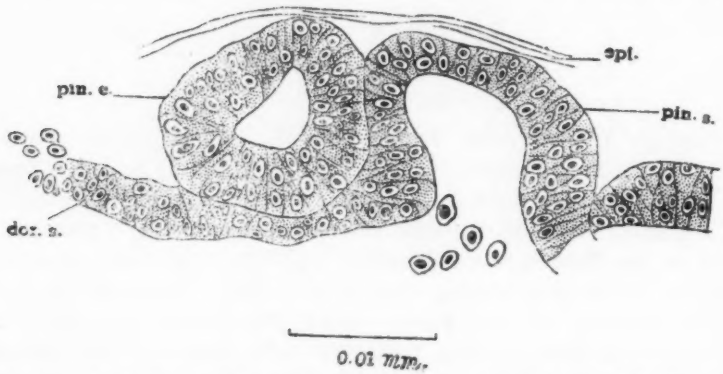
Material and Methods

The material for this paper was collected in Nagpur during July to September 1944, and was fixed in Bouin's fluid; sections of the heads of embryos were stained in Hæmatoxylin and counterstained in Eosin.

[1] *Die Arten der Saurier*, 1872, p. 72.

Description of Stages

The earliest stage in my collection (head length 3·5 mm.) shows the two Pineal vesicles lying one behind the other over the roof of the forebrain (Text-Fig. 1). Of these vesicles, the anterior (*pin.e.*) is completely closed on all sides and is situated slightly towards the right side of the median



Text-Fig. 1.—Sagittal section through the head of a young embryo of *Calotes versicolor* (Daud). *dor.s.*, dorsal sac; *epi.*, epidermis; *pin.e.*, pineal eye; *pin.s.*, pilnea sac. ×3305.

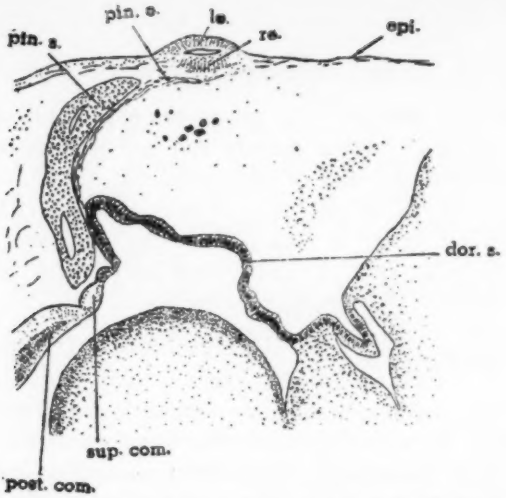
sagittal plane. It is oval in shape with the upper surface slightly flattened. It is covered above by the epidermis (*epi.*) of the head, and behind it lies the posterior vesicle (*pin.s.*) and below it, the wall of the Dorsal Sac (*dor. s.*). This vesicle will ultimately develop into the Pineal eye.

The posterior vesicle (*pin.s.*) is a finger-shaped structure arising from the forebrain. It is hollow and is in communication with the cavity of the forebrain. In front of it is the anterior vesicle (*pin. e.*) with which it does not communicate. Subsequently it will give rise to the Pineal Sac. The wall of the brain just in front of the Posterior Vesicle and below the anterior one will form the Dorsal Sac.

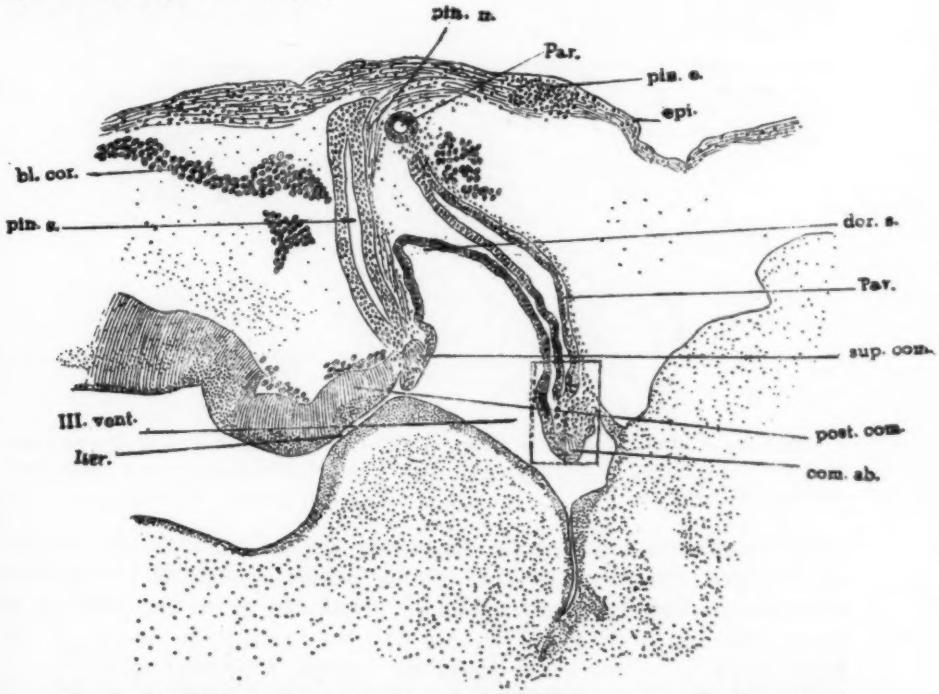
Histologically, both these vesicles resemble the wall of the brain from which they arise.

In the next stage (head length 4 mm.) (Text-Figs. 2 and 3), the pineal complex is more fully developed, and the pineal eye appears to be fully formed (*pin.e.*), its upper portion developing into a lens (*le.*) and the lower portion into retina.

The mesoblastic tissue between the brain roof and the epidermis separates the pineal eye from the forebrain and the pineal eye has apparently



TEXT-FIG. 2.—Median sagittal section through the head of an embryo of *Calotes versicolor* (Daud.) of approximately 4 mm. head length, in the region of the pineal eye. *le.*, lens; *post.com.*, posterior commissure; *re.*, retina of the pineal eye; *sup.com.*, superior commissure (the other lettering as in Text-Fig. 1).

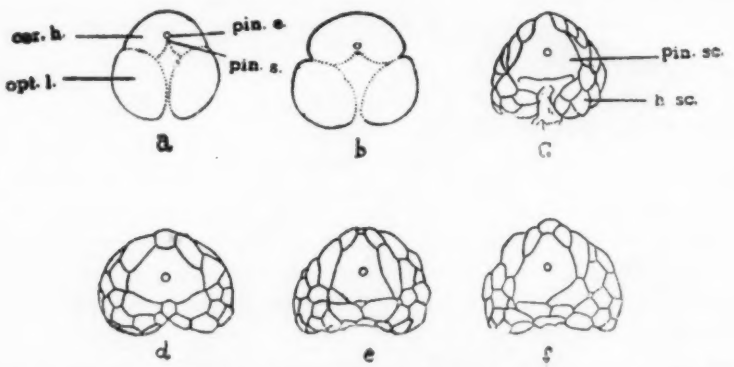


TEXT-FIG. 3. Median sagittal section through the head of an embryo of *Calotes versicolor* (Daud.) of 4 mm. head length. The section is slightly oblique and does not pass through the middle of the pineal eye. *bl.cor.*, blood corpuscle; *com.ab.*, commissura aberrans; *par.*, paraphysis; *III vent.*, third ventricle (other lettering as in Text-Figs. 1 and 2).

lost all connections with it. The pineal sac (*pin. s.*) develops as a glove finger-shaped structure bent anteriorly and ending well behind the pineal eye. From the posterior end of the pineal eye, the pineal nerve (*pin. n.*) goes back below the pineal sac anterior to it and enters the superior commissure (*sup. com.*), where it enters Hebenular ganglion. The paraphysis (Text-Fig. 3, *Par.*) also appears to arise from the forebrain as a hollow finger-shaped outgrowth at the posterior extremity of the Dorsal Sac (*dor. s.*). The limit of the Dorsal Sac is defined anteriorly by the paraphysis and posteriorly by the Pineal sac.

From this stage onwards all these organs persist as such and show the same structure. In later stages the pineal eye undergoes certain changes in its shape and in the amount of pigment present in it, other parts do not show any marked change.

While these organs are being formed internally the pineal eye is seen externally from 3·5 mm. head length onwards as a circular dark blue spot (Text-Fig. 4, *a* to *f*, *pin. e.*) situated nearly in the middle of the head in the



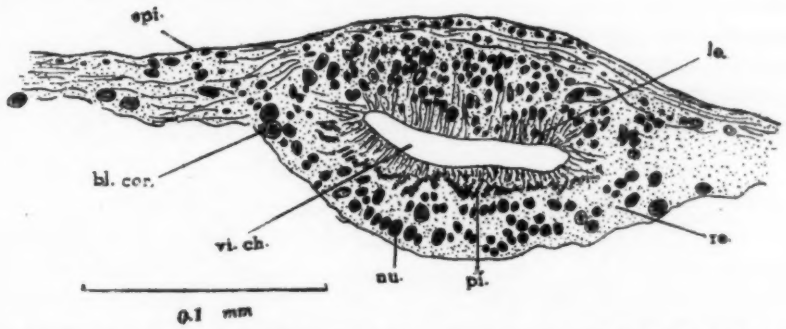
TEXT-FIG. 4.—Dorsal view of the developing embryos of *Calotes* showing developing pineal scale over the pineal eye. *cer.h.*, cerebral hemispheres; *h.sc.*, scales of the head; *opt. l.*, optic lobes; *pin.sc.*, pineal scale.

space bounded in front by the cerebral hemispheres (*cer. h.*) and behind by the optic vesicle (*opt.l*). Immediately behind the pineal eye another blue spot considerably smaller than the pineal eye, marks the position of the anterior end of the pineal sac (*pin. s.*). Very soon scales develop in the head region (*sc.*). Above the pineal eye a large transparent median scale, polygonal in shape, and completely free from pigment, makes its appearance (*pin. sc.*). This pineal scale persists as such in the adult.

Discussion

The Pineal Eye.—The earliest appearance of the pineal eye is shown in Text-Fig. 1. It comprises of the anterior vesicle (*pin. e.*). No other differentiation in the wall of the vesicle such as into an upper lens and a lower retina is visible although the vesicle is completely separated both from the posterior vesicle and the brain roof. The differentiation of the anterior vesicle into lens and retina, therefore appears to occur at a later stage. *Calotes*, in this respect, differs from *Lacerta* (Nowikoff, 1910), and *Sphenodon* (Dendy, 1911), in both of which the upper wall of the anterior vesicle thickens quite early, sometimes long before the two vesicles are constricted off from the brain and from each other.[2] A comparison of Text-Fig. 1 with Figs. 30, 31, Plate 5 of Dendy, 1911 and Fig. 6, Plate 3 of Nowikoff, 1910, clearly shows the difference. Both in Nowikoff's and Dendy's figures the upper wall of the anterior vesicles has thickened to form the lens, but in *Calotes* embryos of roughly the same developmental stage no signs of differentiation are visible as yet in the anterior vesicle.

There is a considerable gap between the first stage (head length 3·5 mm.) and the second stage (head length 4 mm.); the latter represents a more advanced condition (Text-Fig. 5).



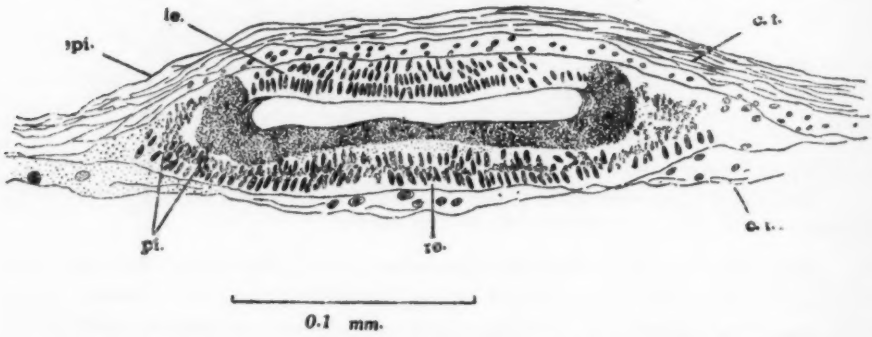
Text-Fig. 5.—Transverse section through the pineal eye of a *Calotes* embryo of 4 mm. head length. *nu.*, nucleus; *pi.*, pigment; *vi.ch.*, vitreous chamber.

The lens is formed from the upper portion of the wall of the anterior pineal vesicle (Text-Fig. 1, *pin.e.*) the region which is in direct contact with the superficial epiblast of the head. The cells of the pineal eye in this region

[2] "The differentiation of the wall of the optic vesicle into lens and retina takes place at a remarkably early stage of development. It may commence even before the two pineal outgrowths have separated from one another which occurs at stage 0 (fig. 30.)"—Dendy, *Phil. Trans. Roy. Soc. London*, 1911, **201**, 265.

become elongated and consequently the wall is thick (Text-Fig. 5, *le.*). At this stage the area of the lens in proportion to that of the retina is very small, the wall of the lens is thickest in the middle but gets thinner as it approaches the retinal region. The lens thus has a biconvex shape at this stage. The cells of the lens are lenticular and they contain elongated fusiform nuclei. No pigment was observed in the lens at any of the stages. There is no sharp constriction at the junction of the lens with the retina and the lens can be distinguished from the retina by its elongated cells and absence of pigment in it. Between the epidermis of the head (Text-Fig. 5, *epi.*), and lens there is a clear and transparent mass of connective tissue (*ct.*). In later stages when the pineal eye becomes dorsoventrally compressed and consequently flattened the biconvex structure of the lens becomes less marked and the area of the lens also increases.

The retina of the pineal eye consists of the usual elements found in other Lacertileans. In 4 mm. head length embryo the wall of the retina adjoining the vitreous chamber is almost free from nuclei (Text-Fig. 5). This area is packed with dark brown pigment granules (*pi.*). The outer region of the retina is limited by a basal layer of cells with nuclei roughly arranged in a single row (*nu*). Between the outer basal layer and the inner pigmented region are numerous irregularly scattered nuclei belonging to pigment cells, the ganglion cells, and other elements of the retina. The vitreous chamber (*vi. ch.*) which is quite conspicuous does not appear to contain any structures in it such as are found in *Sphenodon* and *Lacerta*. In the 6 mm. head length stage (Text-Fig. 6), the pineal eye appears more



TEXT-FIG. 6. Transverse section through the pineal eye of a *Calotes* embryo at 6 mm. head length. *c.t.*, connective tissue; *c.t.f.*, connective tissue fibre.

compressed dorsoventrally and the vitreous chamber becomes narrower and more elongated. The amount of pigment in the retina increases

considerably (Text-Fig. 6, *pi.*) and a thick mass of pigment occupies nearly half of the region of the retina adjoining the vitreous chamber. The nuclei of the retina are more regularly arranged. Besides the inner compact mass of pigment, scattered pigment granules also occur throughout the retina (*pi.*).

Pineal sac.—The posterior vesicle (Text-Fig. 1, *pin. s.*) already described, develops into the pineal sac. Beginning as a hollow finger-shaped outgrowth arising from, and in communication with, the cavity of the forebrain the pineal sac becomes progressively elongated as the epidermis of the head separates from the roof of the forebrain owing to the development of the mesoblastic tissue (Text-Figs. 2 and 3). The pineal sac from 4 mm. head length stage onwards retains a uniform structure. It originates towards the posterior end of the dorsal sac, runs upwards and then onwards and ends slightly behind the pineal eye. In very early stages the anterior end of the pineal sac can be seen as a tiny blue spot just behind the pineal eye in the dorsal groove of the head (Text-Fig. 4 *a*, *b*, *c*, etc.). In advanced stages, however, the pineal sac becomes more deeply situated and hence cannot be seen externally. Originally the cavity of the pineal sac is continuous with the third vesicle (Text-Fig. 1), but this connection is obliterated when the superior commissure (Text-Figs. 2 and 3, *sup. com.*), and posterior commissure (*post. com.*) come together and abut against each other. The original place where the cavity of the pineal sac opened into the cavity of the brain is clearly seen in advanced stages as a groove between the posterior and superior commissures (Text-Fig. 4). The connection of the pineal sac with the brain-roof is however retained through the proximal solid end of the pineal sac. The pineal sac is a hollow structure although its cavity appears to be obliterated in the bend.

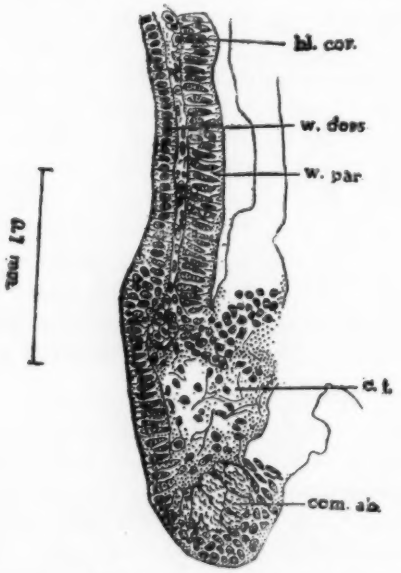
The pineal sac is a hollow structure although its cavity appears to be obliterated in the bend. It is a multicellular thick-walled structure. The cells are more or less irregularly arranged. In general, its structure resembles that of the retina of the pineal eye. In many embryos, presence of pigment was observed in the distal extremity of the pineal sac. Dendy (1899 *b*) has recorded the presence of pigment near the distal extremity of the pineal sac in an embryo of *Sphenodon*. Dendy (1911) also described the presence of pigment in the distal extremity in adult *Sphenodon*.

The Pineal Nerve.—In the earliest stage, the pineal nerve is not seen. At 4 mm. head length the pineal nerve is quite distinct (Text-Figs. 2 and 3, *pin. n.*). It enters the pineal eye towards the posterior region and not in the middle. Traced backwards from the pineal eye the nerve runs at first

nearly parallel to the brain and it bends near the distal extremity of the pineal sac, and runs for the rest of its course in close connection with the pineal sac, anterior to it. The pineal nerve is seen finally to enter the superior commissure beyond which its fate could not be traced.

The Dorsal Sac.—The roof of the forebrain just above the optic thalamus forms the Dorsal Sac (Text-Figs. 2 and 3, *dor. s.*). Posteriorly the sac ends near the base of the pineal sac in the superior commissure (Text-Figs. 2 and 3, *sup. com.*) and its anterior limit is marked by commissure aberrans (*com. ab.*). The dorsal sac is a broad-based triangle in communication with the third ventricle. It is covered anteriorly by the paraphysis (Text-Figs. 2 and 3, *Par.*) and posteriorly by the pineal sac (Text-Figs. 2 and 3, *pin. s.*).

The Dorsal Sac is a thin-walled structure consisting of a single row of cubical cells with small, more or less spherical, nuclei (Text-Fig. 7, *w. dors.*).



TEXT-FIG. 7.—A magnified figure of the region enclosed within the rectangle in Text-Fig. 3 to show the structure of the wall of the paraphysis and the dorsal sac. *w.dors.*, wall of dorsal sac; *w.par.*, wall of the paraphysis.

The nuclei are regularly arranged and placed towards the inner side of the wall.

Paraphysis.—The paraphysis occupies the same position in the embryos of *Calotes* as described in the embryos of *Sphenodon* and *Lacerta*, but its

structure in *Calotes* embryo is less complicated. It develops as a thin finger-shaped process just in front of the dorsal sac (Text-Fig. 3, *Par.*) and communicates with its cavity through the third ventricle. It bends back over the wall of the dorsal sac covering it for the greater part of the length. It runs close and parallel to the pineal sac for a short distance the space between the two being filled with mesoblastic tissue, blood vessels and nerve fibres.

The paraphysis in *Calotes* embryo differs from that in *Sphenodon* (1899 *b*, 1911), and *Lacerta* (Nowikoff, 1910) in the absence of folds and tubules in its walls. Its blood supply also does not appear to be so abundant. The histology of the paraphysis is almost identical with that of the dorsal sac. The wall of the paraphysis is, however, thicker than that of the Dorsal Sac and its nuclei are more elongated and less regularly arranged (Text-Fig. 7, *w. par.*). It does not show the syncytial structure with the nuclei arranged in regular rows as in *Sphenodon* (Dendy, 1910). In *Calotes* embryo the paraphysis has well-defined outlines. As stated above it further differs from other Reptilian embryos in not being a complex tubular structure richly supplied with blood vessels. The paraphysis in almost all *Calotes* embryos examined by me is a simple structure and is neither produced into a convoluted tube nor it is very highly vascular.

ACKNOWLEDGEMENT

This work was done in the Zoology Laboratory of the College of Science as a part of a larger investigation on the development of the pineal organ in the Vertebrates. I am grateful to Prof. M. A. Moghe for valuable guidance.

SUMMARY

1. The various parts of the pineal complex, *viz.*, the pineal eye, pineal sac, pineal nerve, dorsal sac, and paraphysis develop in the same way as in *Sphenodon* and *Lacerta*.

2. The lens of the pineal eye of *Calotes versicolor* appears at a later stage of development than in *Sphenodon* and *Lacerta*.

3. The paraphysis in the embryos of *Calotes versicolor* is a simple structure, neither produced into convoluted tubules nor richly supplied with blood vessels.

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THE EFFECT OF THE INTERACTION BETWEEN IONS, DRUGS AND ELECTRICAL STIMULATION, AS INDICATED BY THE CONTRACTION OF HUMAN UNSTRIATED MUSCLE

By A. K. M. Khan, F.R.C.S., and Inderjit Singh, F.A.Sc.
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Received April 25, 1947

The experiments performed on *Mytilus*, frog, mammalian and avian unstriated muscle (Singh, 1938*a*, 1939, 1940; Singh, Singh and Muthana, 1947) have been performed upon human unstriated muscle for purposes of comparison.

Experimental

The human appendix has been used. The method of stimulation was as described previously (Singh, 1938*a*, 1940). The appendices were removed by one of us (A. K. M. Khan) during abdominal operations at the civil hospital. Immediately after operation, the condition of the appendix as regards its inflammatory condition was noted. In the beginning, a histological study of the sections was undertaken, but later on this was abandoned, as no correlation was found between the response and the histological findings. Further, no correlation was found between the condition of the appendix as noted externally, and the response as our experiments were of a qualitative nature; thus the condition of the appendix was not of any significance in these experiments.

Results

The responses of the human appendix show some fundamental differences from those of unstriated muscle from animals hitherto used.

Effect of temperature.—The optimum temperature in six appendices, two normal and four inflamed, for the response to alternating current, was higher than in the dog's stomach, dog's retractor penis, fowl's gut, or rabit's gut; it was 37–40° C. (Fig. 1). It appears that human muscle acts best when at the body temperature, and a little above which may be encountered in fever; human muscle has therefore, a more restricted range for normal activity. It is possible that for normal functioning, the range of variation allowed in the environmental factors, becomes more restricted with ascent

in the scale of evolution; in other words a more perfect homeostasis is demanded.

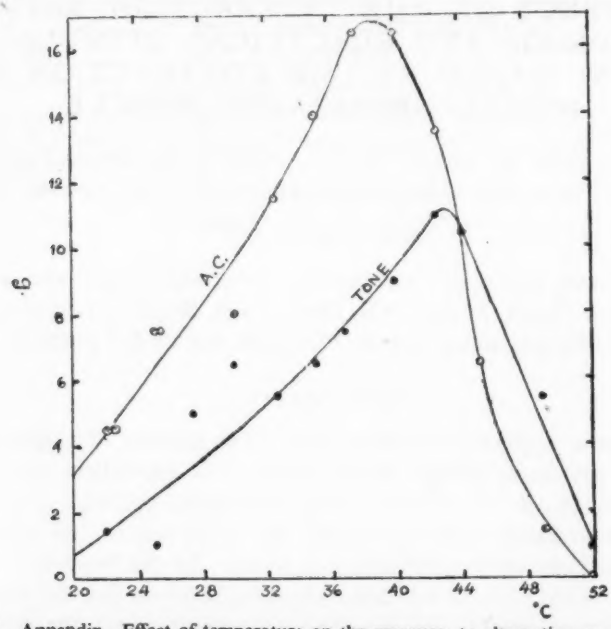

FIG. 1. Appendix. Effect of temperature on the response to alternating current (8 volts for 10 seconds every 15 minutes) and tone.

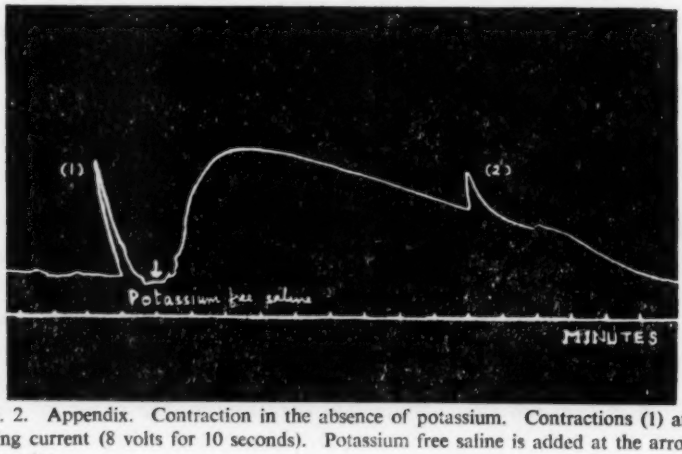

FIG. 2. Appendix. Contraction in the absence of potassium. Contractions (1) and (2) by alternating current (8 volts for 10 seconds). Potassium free saline is added at the arrow. Note the contraction.

The optimum temperature for the response to potassium is the same as that for alternating current; this differs from results on other muscles, in which the optimum temperature for potassium is less than that for alternating current and so less than 37° C.

Other differences.—Human muscle contracts in the absence of potassium (Fig. 2). Anions, Br, I, NO_3, SCN, and drugs eserine, acetylcholine, in small concentrations cause contraction, which is not antagonistic to alternating current (Fig. 3). These results have occasoinally been obtained on other muscles. Higher concentration of the above substances may be antagonistic to alternating current.

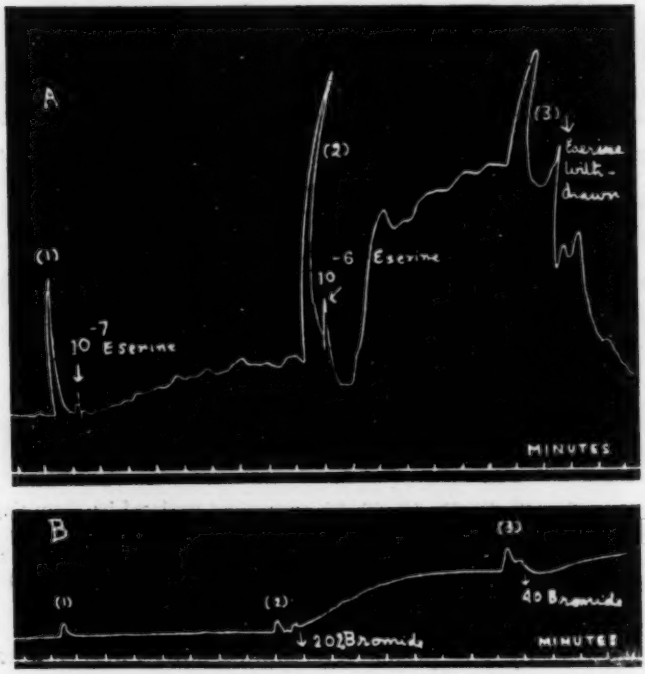

FIG. 3. Appendix. A. Contraction (1) is constant response to alternating current in saline. 10^{-7} eserine sulphate added at first arrow; increase in tone as well as the response to alternating current (2). Addition of 10^{-6} eserine sulphate at second arrow greatly increases tone, but the response to current decreases (3); eserine is then withdrawn and the muscle relaxes at third arrow. B. First two responses to alternating current in saline. 20 per cent. of chloride is then replaced with bromide; tone increases as well as the response to current (3).

Effect of ammonium.—Ammonium initially produces an inhibition to which the muscle adapts. Increase in the concentration of ammonium produces further inhibition and adaptation; this can be repeated till adaptation to inhibition ceases and the muscle finally relaxes (Fig. 4). These

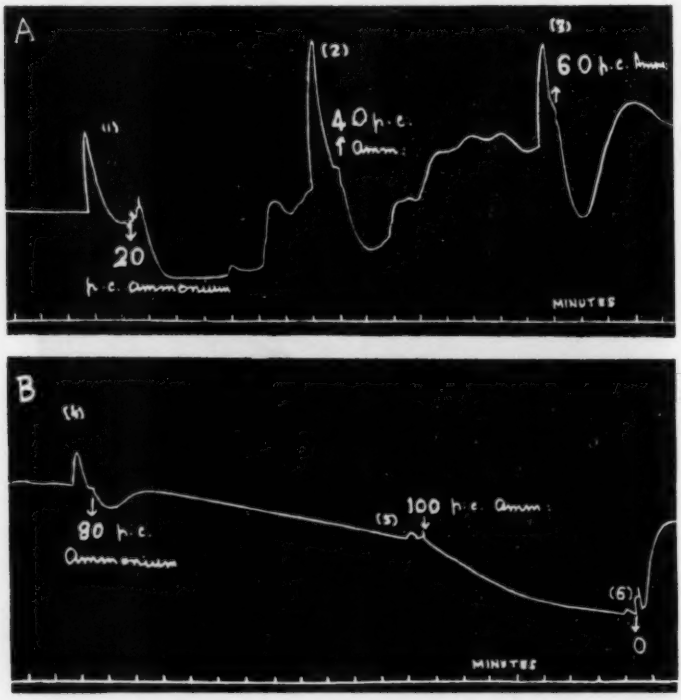

FIG. 4. Appendix. (1), (2), (3), (4), (5), (6) contractions produced by alternating current. 20 per cent. of the sodium of the saline replaced by ammonium after (1); note inhibition from which the muscle recovers. Similarly increasing concentration of ammonium added after each contraction, produces inhibition from which the muscle recovers. With 80 to 100 per cent. replacement, adaptation diminishes and inhibition becomes permanent.

results resemble exactly those produced on *Mytilus* muscle with contraction by alternating current (Fig. 5) (Singh, 1938*b*). This suggests some intimate relationship between inhibition and exitation (Singh, 1945).

The optimum pH for the response to alternating current is 8 and 7.

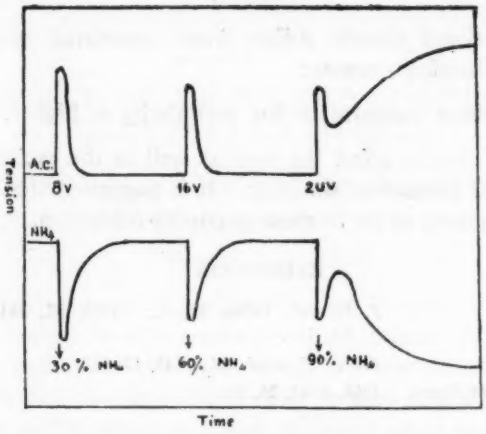

FIG. 5. Diagrammatic comparison of contraction in *Mytilus* muscle and inhibition in the appendix. In the upper curve (*Mytilus* muscle), the voltage of alternating current is increased in steps and in the lower curve (Appendix), the concentration of ammonium is similarly increased.

DISCUSSION

Human muscle thus differs from muscle of lower animals in many important respects. The fact that tone, the response to potassium and that to alternating current may be affected similarly, suggests that in human muscle, adaptation plays the dominant role. This also throws light on the mode of excitation by a substance.

When the muscle is stimulated, the tension produced subsides owing to adaptation. If the excitatory process be termed as V, and adaptation as U, then the tension developed is a function of (V-U). Now, (V-U) can increase in two ways; first, by increase of V, and secondly, by decrease of U. It follows therefore, that excitation may take place by lowering of threshold value of adaptation; the response to potassium and alternating current as well as tone would then be identically affected. If adaptation is due to release of calcium, then excitation would be produced by suppression of ionised calcium in the membranes or elsewhere. Thus increase in calcium would produce inhibition, and decrease, excitation. This is probably the mechanism of surface action by various drugs and ions. The other way of producing excitation is by producing a difference in ionic concentration on the two sides of the muscle membrane.

Summary

Human unstriated muscle differs from unstriated muscle of lower animals in the following respects:

(1) The optimum temperature for excitability is higher, 37°.

(2) Many substances affect the tone as well as the excitability to alternating current and potassium similarly. It is suggested that this is due to decrease of adaptation, as an increase produces inhibition.

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THE ACTION OF DIRECT CURRENT ON UNSTRIATED MUSCLE

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Received April 25, 1947

Winton (1937) found that the stimulation of *Mytilus* unstriated muscle by direct current results in slow relaxation after contraction. He did not find any effect of polarity of the current on the mechanical response. Singh (1938 *b*) found that this tonic contraction or slow relaxation was due to the action of ions in the saline, as it was produced when stimulating ions were present in the saline and was antagonised by agencies that opposed the action of these ions. In striated muscle the mechanical response during fatigue is affected by polarity of direct current: Heilbrunn (1937). Singh (1937) showed that though *Mytilus* muscle may become inexcitable to all forms of stimulation, when the chloride of the saline is replaced with cyanide, it still responds to cessation of direct current.

In the present research, the effects of direct current, which differ in many respects from those of alternating current, were elucidated.

Experimental

The muscle used was that from the frog's stomach; circular strips were used. They were stimulated with direct current by two methods: (1) the first method was that described previously (Singh, 1938 *a*). (2) In the second method the muscle was stimulated by either the anode or the cathode varying from 1·4 to 1·5 volts, using Zn-$ZnSO_4$ non-polarisable electrodes; the indifferent electrode was on one end of the muscle which was killed by heat.

Results

When the frog's muscle is stimulated with direct current, it produces three contractions; one while the current is flowing, the other when the current stops after a latent period of about 2 to 10 seconds, and a third a few seconds (10–60), after the cessation of the current (Fig. 1).

Relation between make and break contractions.—The make and break contractions may be affected identically or oppositely. When they are affected oppositely, their magnitude bears an inverse ratio, so that the total tension may approximately remain constant (Fig. 2). Thus substances that

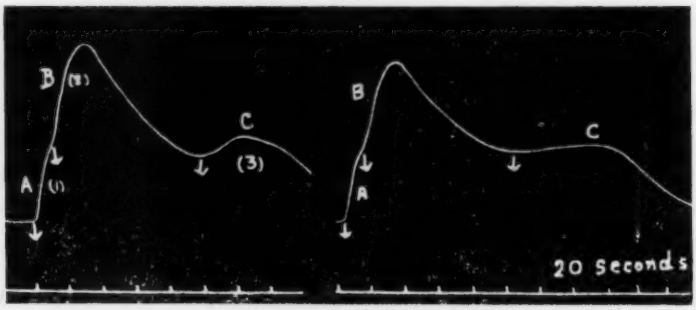

FIG. 1. Frog's stomach muscle. Contraction by 14 volts, direct current (D.C.) for 10 seconds. *A*, the make contraction between first two arrows; *B*, the break contraction; and *C*, the third contraction which is usually brought out by eserine (1 in 10^5).

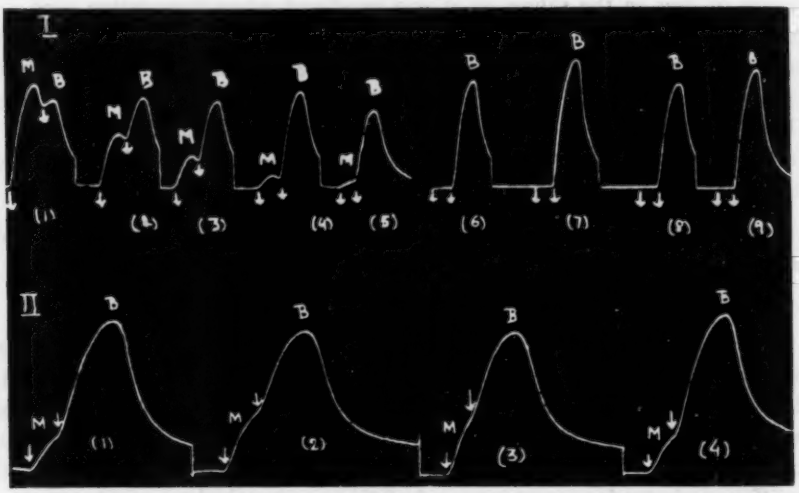

FIG. 2. Frog's stomach muscle. Stimulation by constant current 14 volts for 10 seconds every 10 minutes. Upper curves: 1st 5 contractions in saline, 80 per cent of NaCl replaced with NH_4Cl 6th and 8th contractions in 100 per cent. NH_4Cl. 7th and 9th contractions with the current reversed; note absence of make contraction (M = make and B = break contraction.) Lower curves: 1st and 4th contractions with the current in the same direction. 2nd and 3rd contractions with the current in the opposite direction; the make contraction becomes larger and the break smaller.

increase the make contraction, under such circumstances, will correspondingly decrease the break contraction. With great increase in the make contraction, the break contraction may be almost abolished (Fig. 3). The properties of the make contraction are those of the contraction produced by alternating current and those of the break contraction are similar to those

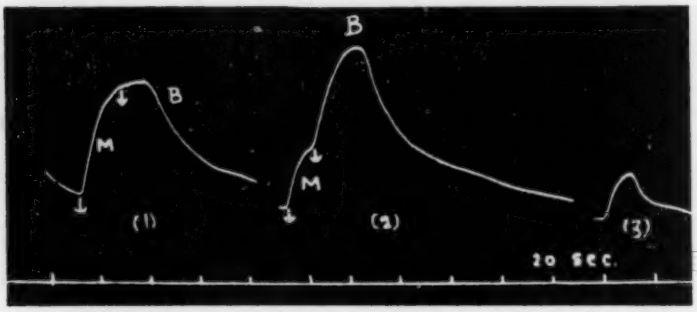

FIG. 3. Frog's stomach muscle. Contraction by 14 volts D.C.;10 sec. First contraction in acetylcholine (1 in 10^6); the break contraction is very small. The second contraction with the current reversed. The third contraction is in adrenaline (1 in 10^6).

of the potassium contraction (Singh, 1938 *a*). If the make contraction decreases, then the break contraction may increase, so that as the former vanishes, the latter alone remains. Sometimes both the contractions are absent and only the third contraction is obtained. This produces a curious phenomenon in that the muscle remains quiescent during the passage of the current, but contracts after the lapse of a few seconds or minutes. Such a contraction is best observed if spontaneous contractions are absent. The contraction is antagonistic to that produced by alternating current. It is akin to the secondary contracture (Singh, 1938 *a*), or is possibly due to nerves (Singh and Singh, 1947). This reminds one of the long latent periods of gastric or pancreatic secretion to vagus stimulation.

Effect of stimulation.—During the beneficial effect of contraction, the make contraction increases, and the break contraction decreases. To begin with, the muscle may be inexcitable to make but may give a large response to break of the current. During fatigue the make contraction decreases, and the break contraction increases. During both these phases, these contractions may be affected indentically. This shows that during fatigue and the beneficial effect of contraction, factors arise which affect the two excitabilities in the same and in the opposite direction respectively.

Effect of strength of stimulus.—With some increase in voltage, at first, both the contractions increase, but thereafter the make contraction increases at the expense of the break, so that with high voltages (40–50 volts D.C.), the break contraction may be abolished (Fig. 4). The latter contraction may, however, reappear, if the response now begins to decline with increase in voltage. The above observations suggest that some common factor which is probably ionic, is responsible for both the contractions, so that if it is

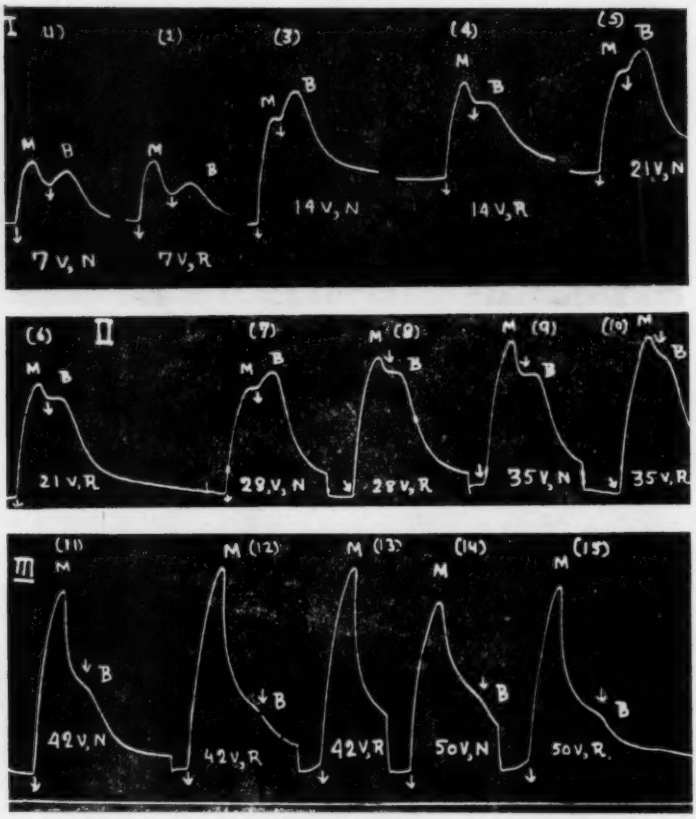

FIG. 4. Frog's stomach muscle. Contraction by D.C. M = make contraction, B = break contraction, N = normal direction, R = reversed direction. Note that in the lowest figure, in the thirteenth contraction with 42 volts, the break contraction has been abolished.

used more for the make contraction, less of it remains for the break contraction.

Effect of temperature.—The optimum temperature for the make contraction is 25 to 30° C. and that for the break contraction 15 to 20° C. Thus the break contraction belongs to the potassium group like the alternating current off-contracture.

Effect of ions and drugs.—The effect of following substances was tested: (1) Monovalent cations, Li, Na, NH_4, K, H. (2) Divalent cations, Ca, Sr, Ba, Mg. (3) Monovalent anions, Br, NO_3, I, SCN, CN. (4) Drugs, adrenaline, acetylcholine and eserine. The effect of these substances on the

make contraction resembles that on the contraction produced by alternating current, and the effect on the break contraction, that on the potassium contraction. The effects of ammonium and potassium are interesting. If all the sodium of the saline is replaced with ammonium or if 20–40 per cent. is replaced with potassium, then the make contraction disappears and only the break contraction remains, suggesting that the latter is dependent upon ions in the saline. All of the sodium may be replaced with potassium, and the muscle still responds to the break of the current (Fig. 5). This suggests

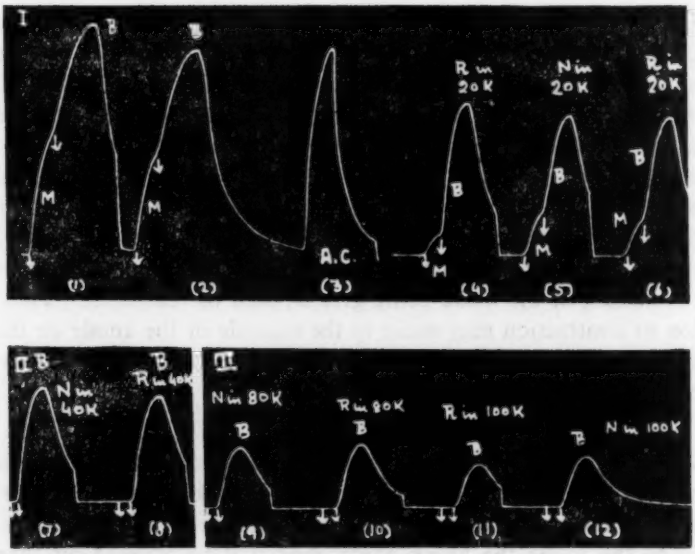

FIG. 5. Frog's stomach muscle. Stimulation by D.C. 14 V/10 sec. I. First two contractions with curves in normal and reversed direction; 3rd contraction by alternating current 8 V/10 sec. for comparison. 4th, 5th and 6th contractions in saline with 20 per cent. of sodium replaced by potassium (R in reversed direction, and N in normal direction). II and III are self-explanatory. K = percentage of potassium replacing sodium of the saline. In curves 7 to 12 the make contraction is absent.

that the inexcitability in excess of ammonium and potassium is not due to damage but to some redistribution of ions in the muscle. Hydrogen ions too (pH 5) produce a similar action. Similarly all the sodium chloride of the saline can be replaced with calcium and strontium chlorides; barium produces similar effect if 20 per cent. of sodium is replaced. Magnesium, on replacing all the sodium chloride, and adrenaline (1 in 10^7–10^6) have opposite action; the make contraction remains but the break contraction disappears.

Effect of polarity of current.—The magnitude of the total response and that of the individual make and break contractions, varies with the directions of the current. The effect on the make and the break contraction is reciprocal. If there is no appreciable difference between the responses when the current is reversed, it can be produced by altering the ionic content of the saline either by replacing the sodium or the chloride ion with some other ion, or altering the total concentration of ions by altering the sodium chloride content of the saline. Polarity may affect the response in the electrolyte-free medium.

Effects of polar stimulation.—On make and break of the current, contractions occur both at the anode and the cathode, the anodal contraction at the make being smaller than the cathodal. The properties of the make contractions are similar to those of the contraction produced by alternating current, and those of the break contraction are similar to those of the potassium contraction. In excess of potassium, as the make contractions disappears, the break contraction becomes powerful, the anodal contraction being the biggest. If contracture is first induced in frog's muscle, or in the guinea pig's uterus, then inhibition is produced instead of contraction, the anodal inhibition at the make being greater than the cathodal. Thus either inhibition or contraction may occur at the cathode or the anode on make or break of the current; this is in agreement with recent work on nerve (see Wiggers, 1944).

Discussion

The contraction produced on break of the direct current depends upon two factors: (1) Changes in the saline. (2) The make response. The make and the break contractions are affected reciprocally, so that if any substance is added to the saline the break contraction will be affected by change in the saline as well as by change in the make contraction. Hence the response is rather irregular.

The break contraction is similar to the alternating current off-contracture. It can be produced in the electrolyte-free medium, so the assumption that it is due to the leakage of ions from the fibres is justified. It is also increased by ions outside the muscle fibres, so that it is produced by leakage of ions into an outer zone (Singh, 1944).

The muscle may be inexcitable to all forms of stimulation except that due to break of a constant current; this suggests that one of the factors that causes diminution in excitability is some rearrangement of ions. This cannot be explained on the assumption that the interior of the muscle fibres is uniform, and that they are surrounded by only one membrane.

The fact that the contraction on make of the constant current, is antagonistic to that on break, is not explained by the current theories of excitation by constant current. The fact that the response varies with polarity, suggests that the membranes of the muscle are not equally permeable in both directions. This would produce rectification, and so account for the stimulating action of alternating current.

Summary

(1) The properties of the contraction produced by break of a constant current are similar to those of the alternating current off-contracture; the make contraction resembles that produced by alternating current.

(2) The muscle responds to break of a constant current when it may be inexcitable to all other forms of stimulation; it may respond when all the sodium chloride of the saline is replaced with chlorides of lithium, ammonium, potassium, calcium, magnesium and strontium or in acid solutions (pH 5).

(3) Magnesium and adrenaline abolish the break contraction.

(4) The response differs with polarity of the direct current; this suggests that the permeability of the membranes is different in the two directions. Stimulation by alternating current is probably due, therefore, to rectification.

(5) The make and the break contractions bear a reciprocal relation to each other.

(6) With polar stimulation, the results are very complicated; contraction or inhibition may occur at the anode or the cathode on make or break of the current.

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STUDIES ON THE EMBRYOLOGY OF MICROCHIROPTERA

Part I. Reproduction and Breeding Seasons in the South Indian Vespertilionid Bat – *Scotophilus wroughtoni* (Thomas)

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Received March 18, 1947

(Communicated by Prof. M. A. Moghe, F.A.SC.)

(With Two Plates)

Introduction

This is the first of a series of papers on the embryology of Microchiroptera. It attempts to record the breeding seasons of one of the species of Insectivorous bats—*Scotophilus wroughtoni* (Thomas), collected at a place about 18 miles from Bangalore (South India). The climatic conditions of this place do not vary much during the year, the place being in the tropical zone. The present paper does not attempt to describe in detail the histological changes which occur in the reproductive tract of the bat during breeding and non-breeding seasons. This will be dealt with in a subsequent paper. It attempts only to record the salient features of the cyclical changes as observed in different months of the year and other phenomena of special interest in relation to the breeding habits of the bat. The work was based entirely upon the collections of wild specimens since the taming of these bats in the laboratory was found to be impossible. Captivation and consequent domestication of these bats might have considerably impaired the normal sexual rhythm, and might thus have given misleading results.

Historical

The subject of the reproductive process of bats has engaged the attention of many workers for over a century. A review of the literature dealing with the reproduction of the Insectivorous bats has been made by several authors. One such attempt was made by Duval (1895 *a*) who reviewed all the earlier work. Later a good summary of the literature was given by Hartman (1933). Baker and Bird (1936) in a paper on the "Seasons in a Tropical Rain-forest (New-Hebrides). Part 4.—Insectivorous bats" gave a short resumé of the work done on the reproductive cycle of the Insectivorous bats. At the time when Baker and Bird published their paper they were

almost the pioneer workers on the tropical species of microchiroptera. Since then quite a large number of workers have described the reproductive process in bats, not only of the temperate and cold climates but of the tropics also. Particular mention must be made of the valuable work done by Harrison Matthews (1937) and Mary J. Guthrie (1933) on the European, South African, and American bats. Harrison Matthew's record of the breeding seasons of the South African bats, though based on a very imperfect collection, gives a fairly clear idea of the sexual rhythm of the tropical bats.

Pagenstecher (1859) was almost the first to notice that there was something peculiar about the breeding habits of the bats. Working on *Pipistrellus pipistrellus* in Germany, he noticed that in winter the uterus of the female was swollen, and this swelling was due to the presence of live spermatozoa, though there was no sign of a ripe Graafian follicle in the ovary. He naturally concluded that copulation occurred in the bat earlier than ovulation and the sperms were capable of being stored in the genital tract of the female for a fairly long time, throughout the winter.

Van Beneden (1875) also observed sperms in the uteri of bats, but concluded that fertilisation occurred immediately after copulation and the fertilised ovum remained dormant till the end of winter. Emier (1879 *a* and *b*), however, confirmed the view of Pagenstecher and showed that in *Pipistrellus pipistrellus* and *Nyctalus noctula* copulation occurs late in autumn and the sperms hibernate during winter inside the uterine tract of the female.

Benecke (1879) and Fries (1879), working on a number of species—*Pipistrellus pipistrellus*, *Plecotus auritus*, *Vespertilio murinus*, *Vespertilio nathusii*, *Rhinolophus hipposiderus*, came to the conclusion that copulation occurred before hibernation and the sperms lived in the uterus of the female throughout the winter and ovulation and fertilisation took place during early spring. The young born in the summer do not copulate in the same season. They thus believed that spermatozoa hibernated in the uterus of the female for a period of at least 4½ months.

Later, Rollinat and Trouessart (1895–97) published their classical work dealing with the reproduction of two different species of microchiroptera, *Vespertilio murinus* and *Rhinolophus ferrum-equinum*. They clearly stated that sperms existed in a dormant state throughout the winter for about 4½ months, from October to the beginning of April, and ovulation and fertilisation occurred at the beginning of spring 8–10 days after the bats 'awoke' from their winter hibernation. Further their experiments with hibernating bats indicated that if the 'sleeping' females were brought to a warm room, ovulation, and consequent fertilisation and pregnancy resulted.

Grosser (1903) described a very remarkable phenomenon occurring in *Nyctalus noctula*, wherein copulation occurred very early—in July or August—and at the end of autumn the cervical canal was blocked by an increase in the amount of connective tissue and the sperms were thus stored up in the vagina. Ovulation and fertilisation occurred at the end of March or in early April when the genital canal was found to be free. This phenomenon which was not described in any other species proved beyond any doubt that copulation must have occurred early in winter or late autumn and fertilisation and pregnancy during spring and summer. Such a blocking of the vaginal passage was not noticed in *Vespertilio murinus*, or *Placotus auritus*.

A parallel instance has been recorded by Courrier (1927) in the males of *Pipistrellus pipistrellus* where he noticed that the testes had degenerated at the beginning of winter leaving only the spermatogonia and sertoli cells, and the testes resumed activity only in the next autumn. He had previously observed (1924) that in *Pipistrellus pipistrellus*, the uterine glands were active during winter and believed that the secretion of the glands acted as nourishment for the hibernating spermatozoa. Rendez (1929) working with *Vespertilio murinus* and *Placotus auritus* showed that in the males the testes did neither exhibit spermatogenetic activity during spring nor the epididymis contain any active sperms. He also concluded that fertilisation occurred in spring by stored sperms received during autumn copulation.

Harrison Matthews (1937) working on the British horse-shoe bats—*Rhinolophus ferrum-equinum* and *Rhinolophus hipposideros minutus*, conclusively proved that copulation occurred in autumn and the spermatozoa stored through winter in the vagina fertilised the ovum which was liberated in spring. He writes, " The occurrence of the vaginal plug in the horse-shoe bats gives some evidence on this question in Rhinolophidæ. In specimen taken during the third week of April the vaginal plug was still in position and exactly similar in all respects to that found in the bats up to that date. In addition, there were present, as usual, spermatozoa in the uterine glands and Fallopian tubes. The presence of the plug entirely filling the vagina showed that copulation had been impossible since that plug hardened in the previous autumn. But the particular point of interest was a large and well-developed corpus luteum in the right ovary and the blastocyst was just passing through the uterus. The ovum must, therefore, have been fertilised by one of the spermatozoa stored in the upper part of the genital tract and the spermatozoa must have been deposited in the previous autumn....". "These specimens show conclusively that in the Rhinolophid bats the spermatozoa stored in winter can and do fertilise the ovum in spring, some five

months later." This account of Harrison Matthews is conclusive enough. But it is a matter of regret that the crucial experiment of keeping inseminated bats segregated until spring, and then examining them for pregnancy was not done by him. Further, he did not give any details regarding the condition of the gonads and accessory structures in the males.

From the foregoing account it is evident that the view held by these authors is that copulation takes place during autumn and that the spermatozoa are stored in the genital tract of the female till ovulation and fertilisation which occur in spring. This observation would apply to *Pipistrellus pipistrellus*, *Nictolus noctula*, *Plecotus auritus*, *Vespertilio murinus*, *Rhinolophus ferrum-equinum*, and *Rhinolophus hipposideros minutus*.

The other view that effective copulation, ovulation, and fertilisation take place in spring is equally strongly advocated by various workers with respect to the bats of the cold climates. Some authors regard this as an exceptional phenomenon occurring either in those bats which have failed to copulate the previous autumn or in the young ones in the first year of sexual life. But these researches have so far been confined to the temperate bats only.

Vogt (1881) was the first to notice the occurrence of non-pregnant females of *Vespertilio murinus* and *Rhinolophus ferrum-equinum* in spring. He believed them to have missed copulation in the previous autumn. He does not mention if he observed spring copulation normally occurring in these bats.

Rollinat and Trouessart, though they categorically deny spring copulation, still record the instance one male specimen of *Eptesecus serotinus* which, when brought to a warm room in early February, woke up from hibernation and tried to copulate. Duval (1895) actually observed spring copulation though not under normal conditions. Similar artificial induction of spring copulation was conducted by Zondek (1933) and Caffier (1934).

Hartman and Cuyler (1927) who worked out completely the life-cycle of *Nyctinomus mexicanus* stated that spring copulation was the rule in these American bats. They recorded the occurrence of sperms in the uterine tract of the female only in March and in no other season. Soon after copulation, fertilisation and gestation followed as in any other mammal. However, in a species of *Myotis* (sp.) from the same locality sperms were seen in the uterus of the female during winter.

Apart from the observations of Hartman and Cuyler quite a large amount of circumstantial evidence has been adduced by the supporters of spring

copulation theory in various species. The conclusions were mainly based on a study of the male reproductive organs. Fries (1879) described that throughout winter and spring the male genital apparatus was full of sperms and the accessory reproductive organs were in full swing of activity. Courrier (1927) made a more detailed study on *Pipistrellus pipistrellus* and recorded that the interstitial cells of the testes and the accessory sexual organs were in full activity during winter, though the seminiferous tubules contained no sperms, but only spermatogonia and sertoli cells. Rollinat and Trouessart had also observed the storage of spermatozoa in the epididymis and the bladder of the male during winter. Nakano (1928) also recorded the storage of spermatozoa in the epididymis throughout winter. There has, however, not been any conclusive proof that fertilisation occurred by the spermatozoa stored in the genital organs of the males during winter.

Caffier and Kolbow (1931), though they accepted the possibility of fertilisation being effected by the spermatozoa received in autumn copulation, they made the startling discovery that the testes showed spermatogenesis not only in November but also in March in many species such as *Pipistrellus pipistrellus*, *Plecotus auritus*, *Barbastella*, *Eptesecus*, *Myotis*, *Rhinolophus hipposideros*, etc.

The only conclusive evidence in support of effective spring copulation was given by Mary J. Guthrie (1933) who observed the normal occurrence of only spring copulation in many species of North-American insectivorous bats.

There is thus a vast amount of literature available regarding the breeding habits of the insectivorous bats inhabiting temperate and cold climates. Unfortunately there is no complete account of the breeding cycle of the tropical species of microchiroptera, and the little knowledge we have is derived only from records of pregnancy. With regard to the copulating season there is practically no information except for the casual observation of the occurrence of visible secondary sexual characters in different seasons in the males of a few species of microchiroptera. Braestrup (1933) recognised in two males of *Chærophon pumilus* in tropical Africa a large crest of hair on the neck and back and that the scrotum was swollen. This season varied at different places. Thus no generalisation could possibly be made regarding the exact season of copulation and sex cycle on such meagre and casual observations.

A fairly clear account of the breeding seasons of *Miniopterus australis* was given by Baker and Bird (1936). They noticed that, " Conception in this species occurred in the beginning of September....". " And the young

were probably born in the second half of December, and the duration of gestation is about 110 days". Further in the middle of August (11th) the examination of the uteri for sperms gave negative results there being no sperms in the uteri or uterine glands. There was a large Graafian follicle with much liquor folliculi in the ovary and there were signs of early meta-œstrus condition of the uterine glands. The authors recorded that, "Presumably insemination and ovulation would have taken place two or three weeks later".

Their examination of the male specimens substantiated the results of the examination of the female. An abundance of spermatozoa in the epididymis was seen in July, August, and September. From October onwards there was a decrease in the spermatozoa and till May next the epididymis was practically empty. They therefore observed, "One sees clearly that copulation takes place about the end of August and the development of the embryo starts at once. Copulation occurs at a time of the year, when the days are beginning to get longer and the temperature is rising, *i.e.*, in spring"....."Thus *Miniopterus australis* falls into the same category as *Nyctinomus* at Texas, which Hartmann and Cuyler (1927) showed to copulate only in the northern spring".

After the classical work of Baker and Bird there has been very little work on the tropical bats. Harrison Matthews (1941) in his paper 'On the genitalia and reproduction of some South African bats' summarised that in the tropical species there was nothing comparable to the winter hibernation of the temperate bats. Only in the case of *Miniopterus dasythrix* he mentions "Possibly that impregnation had taken place weeks, or even months, previously and that spermatozoa had been stored in both sexes as in some European bats". However, in the "summary", he observed, "for early in July the females were pregnant with only blastocysts, indicating mating at a season corresponding with the earliest beginnings of the southern spring. It is of course possible that insemination took place in the preceding autumn and that fertilisation or development had been delayed, as in some bats of the temperate regions, but it does not appear to be likely because there is no evidence that this species hibernates".

We thus see that though a great controversy exists regarding the exact seasons of copulation and fertilisation in the insectivorous bats of the temperate and cold climates, there seems to be entire agreement among the workers on the tropical bats, that copulation occurs in early spring and is immediately followed by fertilisation and gestation.

My study of the reproduction of the South Indian Vespertilionid bat *Scotophilus wroughtoni* (Thomas) confirms the above view. There is no storage and hibernation of spermatozoa.

Material and Methods

Bats of this species were collected round about Bangalore from the forests of Hoskote (about 17 miles east of Bangalore). Some were also collected at Seringapatam about 75 miles west of Bangalore. This does not alter the results of the work as both the localities conform to the same plan of breeding. *Scotophilus wroughtoni* is essentially an arboreal species living inside hollows of large trees. A few excursions were made to the caves and dungeons near Bangalore and Seringapatam, but at no time could we collect a single specimen of this species though many other species were collected in large numbers. Further, it appears that this particular bat is always found to live in association with two other species of insectivorous bats—*Scotophilus temminki* and *Taphozoas longimanus*, for all our collection of bats included all the three species.

Scotophilus wroughtoni is a fairly large bat with a brown coloured belly and the back of darker hue. The tail projects slightly beyond the interfemoral membrane. The bats hang down from projections inside the hollows of trees. They were caught by using a net. They are ferocious and are to be handled with care, as they bite otherwise.

The specimens were killed by chloroform and immediately dissected and the genital structures removed. The carcases are all preserved in formalin for further study. The reproductive organs were fixed in various fluids. But Bouin's picro-formal gave the best results. After fixation the material was transferred to 70% alcohol. In the females the mammary glands and in the males the adrenal bodies were similarly fixed. Serial sections of the ovaries, Fallopian tubes, the uterus and vagina were taken in all cases where there was no visible signs of pregnancy.

An account of the changes in the male reproductive and detailed histological œstrous changes in the female will be dealt with in the next part.

Collections of bats began in the month of May 1945 and is still being continued to the present day. Attempts were made to collect as many times as possible in all months of the year to complete the data regarding the breeding habits.

Table I gives the record of the collection of the bats so far made.

TABLE I

Scotophilus wroughtoni (Thomas): *Monthly record of collections*

Month	Males	Females	Pregnancy in Female
January ..	3	5	Non-pregnant
February ..	5	3	,,
March ..	2	4	Early morulæ
April ..	2	40	Early blastocysts
May ..	4	30	Advanced pregnancy
June ..	2	2	Full term
July ..	..	..	..
August ..	..	..	..
September ..	1	1	Non-pregnant
October ..	1	1	,,
November ..	1	2	,,
December ..	2	17	,,

The above numbers do not probably indicate the sex-ratio because in practically all our collections the females outnumbered the males. One thing worth recording is that during the months of January and February there were a greater number of males than during the other months. This fact taken with the other things might probably indicate that during this period only do the males and the females live together, while at other periods males live segregated from the females. No definite generalisation is, however, possible at this stage.

OBSERVATIONS

(*a*) *Number of embryos in a litter.*—At each pregnancy there are two embryos, and each ovary shows a corpus luteum—a fact which is of very rare occurrence among the microchiroptera, single embryos being the rule. Two embryos were also observed in *Scotophilus temminki*. Occurrence of double embryos was noticed by Ramaswamy (1933) in another Vespertilionid bat, *Vesperugo leisleri* (Kuhl). Harrison Matthews (1942) states, "One of the most interesting characters of the female genitalia in the microchiroptera is the bilateral assymetry which occurs in varying degrees of intensity"...."Most bats, except those of the family Phyllostomatidæ, have a bicornuate uterus, but nearly always bring forth only one young at a birth; consequently as a rule, only one uterine cornu is occupied by pregnancy. It has been found in very many species of different families that there is a constant tendency for the right side of the genitalia to be the functional one. In many European Vespertilionids, although pregnancy can occur on either side, the majority of pregnancy has been found in the right cornu". In *Rhinilophus hipposideros* he has shown that "the left ovary appears to be degenerate and never to produce mature ova, the pregnancy being always

on the right side" (Matthews, 1937 *a*). I have also observed that in many of the species of microchiroptera that I collected there was always a single embryo in the uterus. Thus, *Scotophilus wroughtoni* differs from a majority of the microchiroptera in having two embryos in the litter. However, there is one specimen in my collection in which the *left* ovary shows two masses of corpora lutea and two unimplanted blastocysts in the *left* horn of the uterus but at different levels. Probably double implantation never occurs in this species in the same uterine cornu, the ovum, before or after fertilisation moving into the other cornu for implantation even in those exceptional cases where double corpora lutea occurred in the same ovary. This surmise seems to be correct, because after the establishment of the placenta, there was no case where a double embryo occurred in the same uterine cornu. A similar migration of the ovum from the ovary to the opposite uterine horn for implantation has been described as a normal occurrence in the case of *Miniopterus dasythrix* (Temm.) (Harrison Matthews, 1942).

(*b*) *The breeding seasons.*—Pregnancy records show that the female has a very sharply defined annual breeding season. Pregnancy was observed only from the 22nd March upto about the end of June, and at no other period was a pregnant specimen collected. This seems to confirm the observations of Baker and Bird (1937) on *Miniopterus australis* which " presents a very sharply defined annual breeding season ", where pregnancies occurred only during the months of September, October, November and December, and no pregnancy during the other months of the year. This is also the case in all the species of temperate and cold climates so far examined by various authors. Marshall (1922) states, " it does not appear to be known whether the poly-œstrous condition ever occurs in bats." However, Ramaswamy (1933) observing pregnant uteri in early January in *Vesperugo leisleri* (Kuhl) suggests, " It is also possible that there is another season when these begin to breed "....." It looks as though after a very short anœstrum following the summer gestation the pro-œstrous cycle again commences ending in the copulation of the females in cold weather." Harrison Matthews is the only other author to record a poly-œstrus condition in *Nycteris luteola* (Thos.) and *Nycteris hispida* (Schreb.), wherein he observed pregnancies in lactating bats and concluded, " The quick succession of pregnancies also points to the possibility that this species, unlike all other bats as far as they are known, may be poly-œstrus." But *Scotophilus wroughtoni* without any doubt has only one annual breeding season.

(*c*) *The œstrus and copulation.*—The uterus which shows inactive glands as late as November suddenly springs to activity in February, and the glands

hypertrophy with a definite increase in the vascularisation of the uterine submucosa (Fig. 1). A very careful microscopic examination was made to detect the presence of spermatozoa but in specimens collected in November, December, January and February no sperms were seen in the uterus, vagina, or the Fallopian tubes. The ovaries of the February specimens showed great activity and exhibited a large number of developing Graafian follicles (Fig. 2). Ovulation does not certainly occur upto the 10th of February. Examination of specimens collected on the 24th of March clearly shows that ovulation not only has occurred but in all females early morulæ were present. These were lying loose in the uterine lumen. Further the vagina showed large numbers of degenerating spermatozoa. Another curious fact noticed was that out of the twelve females collected on 1st of April all were pregnant—pregnancy being recognised only after careful microscopic examination of the uterus, and in all the cases the blastocysts were in the same stage of development, lying loose in the uterine cavity (Fig. 3). This fact clearly shows that fertilisation occurred in all the specimens at about the same period, if not on the same day. This, taken along with the fact that the females collected on the 10th of February showed no spermatozoa, indicates that the period of copulation is also very sharply marked, and it must have occurred between the 10th of February and the 24th of March. Judging by the age of the morula on the 24th of March one can easily place the time of fertilisation somewhere about the third week of March. It is, however, not possible to clearly decide whether copulation occurs before or after ovulation because I have unfortunately no collection made during the 1st, 2nd, or the 3rd week of March. But probably copulation might have occurred a day or two after ovulation because no sperms were seen in the Fallopian tubes while sperms were quite abundant in the uterine lumen in the specimens collected on 24th March. There is no instance of a tubal ovum in my collection.

Pregnancy was noticed in all specimens collected between the months of April and June at progressively advanced stages. The June embryos were far advanced in development, though not of full term. Parturition can safely be placed at the last week of June or the first week of July. The period of gestation thus extends from 105 to 120 days.

In the whole of my collection there was no instance of pregnancy in a lactating female.

(*d*) *Age and growth.*—All females captured during April, May and June were pregnant without a single exception. This seems to be a very interesting feature, and Baker and Bird omit to make a mention of this.

Rollinat and Trouessart working on some of the Rhinolophid bats observe "frequently the young females in their second year do not experience œstrus, and consequently their first œstrus does not occur until their third autumn". These authors divided their material into four groups: virgin animals in their first autumn; virgins in their second autumn; animals experiencing their first œstrus, some in their second and some in their third autumn; and parous animals, some in their third and some at least in their fourth autumn" (Harrison Matthews, 1937).

Harrison Matthews (1937) also records, "Young bats do not reach their first œstrus until their second autumn, when they are at least 15 months old. Parous bats will at least reach their second œstrus when they are 12 months older, and at least 27 months of age. By the time when they have weaned their second young one they will be 34 months old". He thus endorses the view-point of Rollinat and Trouessart.

He also tried to determine the age of his specimens by their pregnancy records. He says, "the present series of specimens show clearly that both species of British horse-shoe bats normally do rear a second young one, and consequently must reach an age of at least three years"...."Further on purely theoretical grounds these bats must live to an age of at least four years, because each pair must produce more than two young in its life-time, to allow for wastage, if the species is not to become extinct. Females must therefore produce at least three young each, and accordingly reach an age of four years."

Theorising on similar lines regarding *Scotophilus wroughtoni*, as there is no non-pregnant female during the gestation season, *i.e.*, April to June, and also as there is no record to show that the bats might become pregnant during any other month of the year, as it is moncestrous, the species must get into sexual activity in its first year and become pregnant. Thus, before it has completed its one year of age, it will have given birth to young ones. Furthermore, the growth of the young one is very rapid as is indicated by the ovaries of the specimens collected in February. That the non-occurrence of non-pregnant females during the months of April, May and June, during 1945 and 1946, cannot be an accident. It must be presumed that all females had become pregnant. All these facts clearly indicate that the young born in the month of July the previous year get into sexual activity during next February and March.

Purely on theoretical grounds, and fitting with the conclusions of Harrison Matthews, these bats must produce at least three young ones each, to perpetuate the race, and hence must at least live for two years. As this

particular species under study bears two young ones in each litter it is quite possible the bat becomes pregnant at least twice in its life-time. Further, there is no indication to show that the bat had become pregnant more than twice as revealed by the residual placental discs. This taken along with the fact that the bat experiences an annual breeding season indicates that it must live for atleast two years and that it may not become pregnant a third time. Furthermore, Anderson (1917) states that the length of the period of immaturity will, as a general rule, in some vague sort of way, enable us to form an opinion of the normal age the individual is destined to obtain, a mammal which quickly becomes full grown will probably have a rather short series of years to live as adult, and *vice versa*." Anderson places the age of the bat *Rhinolophus rouxi* " at five or six years as the extreme possible age of the bats ", as calculated from their tooth-wear. Taking all these things into consideration *Scotophilus wroughtoni* does not probably live as long as *Rhinolophus rouxi*, or the British horse-shoe bat, which are supposed to have a longevity of about four and a half to five years but might live, without doubt, up to about three years and probably not more. A more clear and definite figure will be arrived at by examining the tooth-wear on the same lines as Anderson did, which will be shortly undertaken.

Conclusions

Two very important facts are recognised by the study of the reproductive phenomena in *Scotophilus wroughtoni*. In the first place, there is a very sharply defined breeding season confined to about the middle of March, and secondly all the females collected during the months of April, May and June are pregnant. In an unvarying tropical climate the occurrence of an annual monœstrous condition is by itself remarkable, and much more so in the case of a bat which is confined to the hollows of trees—considering the non-conductivity of the wood to temperature, and thereby living under an almost constant environmental condition throughout the year. Baker and Bird (1936) also make a similar discovery in the bats of New-Hebrides and come to an identical conclusion. The seasonal change in the temperature alone does not probably determine the onset of the breeding activity, as was supposed by a few of the early workers on the bats of temperate and cold climates. Furthermore, too much stress cannot be laid on the factor of light and of the lengthening of the day, considering the fact that the bats are essentially nocturnal creatures. Unless experimental data is available regarding the influence of light on the breeding habits of bats, this conclusion cannot be accepted at present.

As copulation is immediately followed by fertilisation and gestation the problem of winter hibernation of the spermatozoa, as occurs in the bats of cold climates, becomes unnecessary.

Summary

1. A study of the literature in the reproduction of bats reveals that there are two types of sexual phenomena exhibited by bats, some bats experiencing a definite hibernation during winter after copulation in autumn, and others where copulation is immediately followed by fertilisation and gestation in spring. The bat *Scotophilus wroughtoni* does not show any evidence of a winter "sleep" and thereby falls into the second category.

2. *Scotophilus wroughtoni* has a sharply defined breeding season, copulation occurring at about the middle of March and followed immediately by fertilisation and gestation.

3. The period of gestation is about 105 to 115 days.

4. The age which this bat attains, as determined by its pregnancy records, may safely be placed at about three years.

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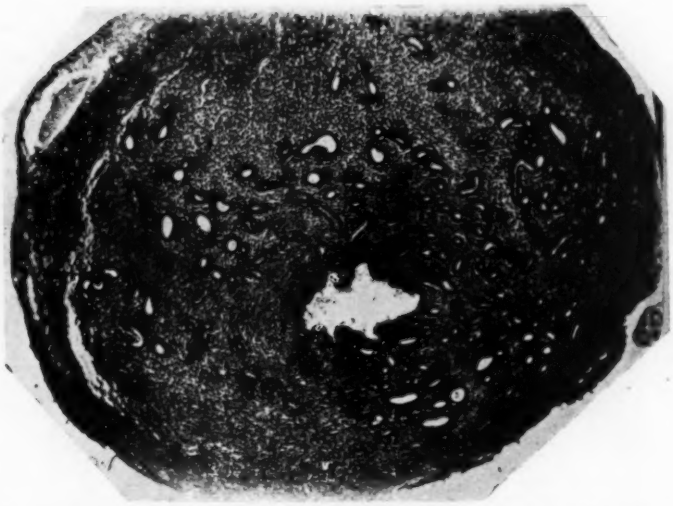

FIG. 1. Transverse section of the uterus of a specimen collected on 10th February showing great hypertrophy of the glands at the onset of the breeding season.

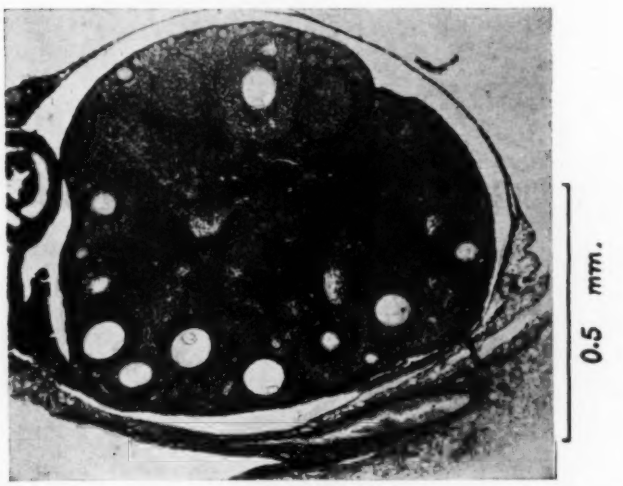

FIG. 2. Transverse section of the ovary from a specimen collected on 10th February showing the great activity of the germinal epithelium. The ovary presents a large number of graafian follicles. Ovulation has not yet occurred.

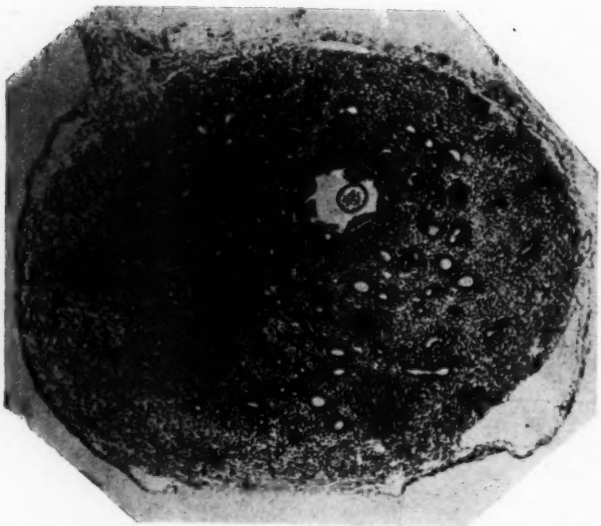

FIG. 3. Transverse section through the uterus of a specimen collected on 1st April showing a free unimplanted blastocyst lying loose in the uterine lumen. All specimens collected on this date show blastocysts in the same stage of development.

UNDESCRIBED MALES OF TWO SPECIES OF GALL MIDGES*

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Received August 22, 1947
(Communicated by Prof. S. G. M. Ramanujam, F.A.SC.)

The present paper deals with the descriptions of males of two species of Indian gall midges (Itonididæ: Diptera). Both the species were first described from female specimens collected by the author from Travancore; the following descriptions are based on males collected on subsequent dates.

The *Allotype* males are to be deposited in the Zoological Survey of India, Benares Cantt.

Trichopteromyia Manii Nayar

The author first described the species (Nayar, 1944) from female midges collected at light from Trivandrum. The following is a description of the male collected from Trivandrum on a later date, at light.

Male.—0·6 mm. long, brownish-red. Eyes confluent above. Palpi quadriarticulate, as in female, scaled; basal segment cylindrical, shortest; terminal segment longest, ovate, more or less pointed distally. Antennæ with fifteen segments, bearing whorls of long setæ, structure more or less similar to that of the female; first segment broadest, hemispherical, the rounded portion attached to the head; second segment globose, slightly compressed, its diameter three-fourths that of the broad end of the first, the attachment to the basal segment at an extra-central point; third a little less in diameter than the second; the stems increasing in size distally; thirteenth segment with a stem as long as the thickness of the third, the enlargement one-third as long as the length of the segment; fourteenth segment with a button-like process representing the fifteenth segment, the enlargement more than two and a half times as thick as the fifteenth. Mesonotum smoky brown. Wings hyaline, as in the female. Halteres blackish brown. Legs smoky-brown, moderately hairy, hindlegs longest. Claws simple, as long as the empodium. Abdomen reddish and dark scaled. Genitalia small; basal clasp segment cylindrical, oblong, three-fifths as broad as long; terminal clasp segment small, sting-like, oblong-ovoid,

* Part of thesis approved for the Ph.D. degree of Travancore University.

slightly longer than the breadth of the basal clasp segment, with a pin-like pointed tip, slightly hairy; style small, rounded at tip, about as long as the basal clasp segment.

Type locality: Trivandrum. Collected at light on various dates in June 1944.

This species differs from the genotype *Trichopteromyia modesta* Williston in the smaller size of the body, the quadriarticulate palpi and the antennal characters.

Prolasioptera æschynanthus-perottetti Mani

Mani (1943) described the species from female specimens bred by me from stem galls on *Aeschynanthus perottetti* A. Dc., from Pampadamparai Hills in the High Ranges of Travancore. Subsequently I was able to rear males also. The following is a description of the male:

Male. 2 mm. long, brownish. Palpi as in the female. Antennæ incomplete, similar to that of the female; third and fourth segments fused together. Mesonotum dark brown. Wings and halteres as in the female. Abdomen comparatively stout. Genitalia (Fig. 1) small; basal clasp segment oblong, its length slightly less than double its own breadth; terminal clasp segment roughly rounded at its joint with the basal clasp segment, malleiform, with fairly serrated and moderately chitinised tip, length about two-thirds that of the basal clasp segment, broadest part about one-third its own length; style as long as the basal clasp segment; dorsal plate deeply cleft and truncated distally.

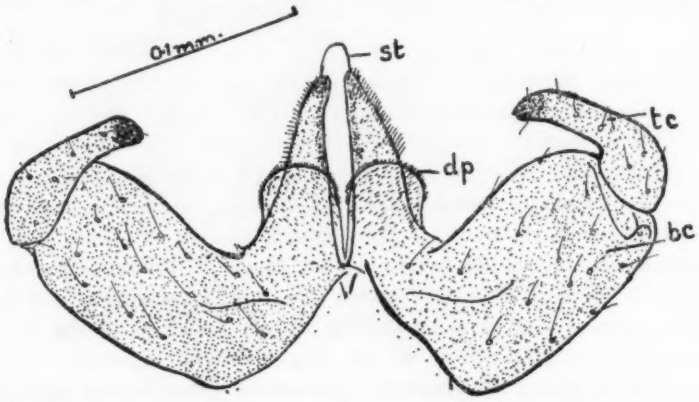

FIG. 1. Male genitalia of *Prolasioptera æschynanthus-perottetti* Mani.
bc, basal clasp segment; *dp*, dorsal plate; *st*, style; *tc*, terminal clasp segment.

Type locality: Pampadamparai Hills in the High Ranges of Travancore. Reared from stem galls on *Aeschynanthus perottetti* A. Dc., in August 1944.

This species is easily distinguished from the other Indian species *Prolasioptera annandalei* Mani, by the longer body and the twenty-one segmented antennæ.

Nayar (1945) described the stem gall on *Aeschynanthus perottetti* A. Dc., from which the midges were bred. A leaf gall has also been collected by him on the same plant, from Pampadamparai Hills, and is believed to be produced by the same midge. I give below the description of the gall:

Leaf gall.—16 mm. long, 5 to 9 mm. across, irregular, green with a violetishbrown tinge, soft, succulent, somewhat granulated inside, hypophyllous, with a number of larval chambers inside.

Locality: Pampadamparai Hills.

ACKNOWLEDGEMENTS

I am very grateful to Prof. M. S. Mani, St. John's College, Agra, for help and guidance in my studies on gall midges and plant galls.

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CYTOGENETICAL STUDIES IN SESAMUM

Part I. Cytology of the Parents, *Sesamum orientale* Linn. and *Sesamum prostratum* Retz. and the Cytology of the Sterile Hybrid between them and of the Fertile Amphidiploid

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I. Introduction

Pedalineæ is a very small family of annual and perennial herbs, distributed mainly in the eastern tropics. Bentham and Hooker (1885) record only two genera in India, *Pedalium* and *Sesamum*. *Martynia* which is also included in this family is reported to be an American weed introduced into India.

The genus *Pedalium* is represented only by a single species, *Pedalium murex* Linn. which grows wild in waste lands of South India. The genus *Sesamum* is represented by both perennial and annual species. Bentham and Hooker (1885) have recorded only three Indian species of *Sesamum*,

Sesamum orientale Linn. and *Sesamum prostratum* Retz., and *Sesamum laciniatum* Klein. Among these *Sesamum orientale* is an annual whereas the other two species are perennials. *Sesamum prostratum* grows wild on the sand dunes near about the shores of Madras while *Sesamum laciniatum* thrives on the barren rocks of the Deccan hills.

Little cytological and cytogenetical data have been recorded for the two genera, *Pedalium* and *Sesamum*. The genera *Pedalium* and *Martynia* have been investigated cytologically and cytomorphologically in this laboratory as part of the extensive cytogenetical investigations in the family Pedalineæ (Srinivasan, A. R., 1942). The chromosome number of *Pedalium murex* Linn. was determined to be $2n$: 16 and its life-history was worked out with special reference to the development of the endosperm haustoria in the female gametophyte. The species *Martynia diandra* Glox, which grows wild in these parts, was also investigated and its diploid chromosome number was determined to be 32.

According to Schnarf (1931) the genus *Sesamum* has been investigated with reference to the occurrence of endosperm haustoria, by Balicka Iwanowska (1899).

Morinaga *et al.* (1929) determined the somatic number of *Sesamum orientale* ($2n$: 26). Nohara (1934), Richharia and Suguira (1936) have reported the meiotic number of the same species (*Sesamum orientale*) to be 13. The present cytological investigation goes to confirm the numbers previously recorded.

The perennial species *Sesamum prostratum* has never been investigated either cytologically or cytomorphologically till recently when its meiotic number was determined to be 16 by Ramanujam (1941). This number has been confirmed in the present investigation.

While cytogenetical investigation in this laboratory had proceeded more than half way through, a short note appeared in *Current Science* recording some data in respect of hybridisation between the cultivated and wild species of *Sesamum* (Ramanujam, 1942).

In the course of the present investigation the chromosome number of *Sesamum laciniatum* Klein, another wild species, was determined for the first time in this laboratory to be $2n$: 28 (Raghavan and Krishnamurthy, 1945).

Interspecific hybridisation between *Sesamum orientale* and *Sesamum prostratum* has been in progress for some years now in this laboratory and the sterile hybrid derived therefrom was made fertile artificially by the induction of amphidiploidy, through the application of Colchicine. The cytology

of the sterile hybrid, its meiotic irregularity and the ultimate formation of abnormal sporads are detailed in this paper. The regular meiosis of the fertile hybrid after the artificial induction of amphidiploidy has also been included.

II. Materials and Method

Crops of *Sesamum orientale* belonging to the local red-seeded strain were raised from time to time in the University Botanical Gardens, Annamalainagar. Seeds of *Sesamum prostratum* were collected from various localities, especially from Adyar beach, Madras and Coimbatore. Seeds were sown in small pots and were kept in a warm room where they germinated early. Root tips from *Sesamum orientale* were available within 60 hours after sowing whereas those of *Sesamum prostratum* could be obtained only after 5 or 6 days.

Good root tips of the parents and the hybrid could easily be obtained without injuring them since they were sown only on the upper layer, just below the soil. Various fixing fluids were used and fixing was done at various intervals of time. Maximum mitotic activity was observed at mid-day. The fixatives used were Karpechenko's modification of Nawaschin's chrome-acetic-formalin, Irene Manton's modification and Muntzing's formula. Of these fixatives, Irene Manton's modification proved to yield good results. Prefixation in Carnoy's fluid was done in all cases to aid proper fixation.

Flower buds were fixed at various hours of the day. Here also mid-day fixing showed good results. Irene Manton's formula was used. Materials were imbedded in paraffin of melting point 52° C. using chloroform as the paraffin solvent.

Sections were cut at thickness varying from 12 to 15 microns and stained in Newton's Iodine Gentian Violet and Haidenhain's Iron Alum Hæmatoxylin (Chamberlain, 1932). Right stages of anthers were determined before fixing by aceto-carmine examination. Drawings were made at table level using Abbe drawing apparatus and their respective magnifications are indicated.

III. Cytological Observations

(*a*) *Sesamum orientale* Linn.

Somatic chromosomes.—Fig. 1 shows a somatic metaphase plate of *Sesamum orientale* with 26 chromosomes thus confirming the previous record made by Morinaga *et al.* (1929). There is no disparity in the size of the chromosomes in the somatic complement. Almost all the chromosomes would appear to be characterised by terminal centromeres. An analysis

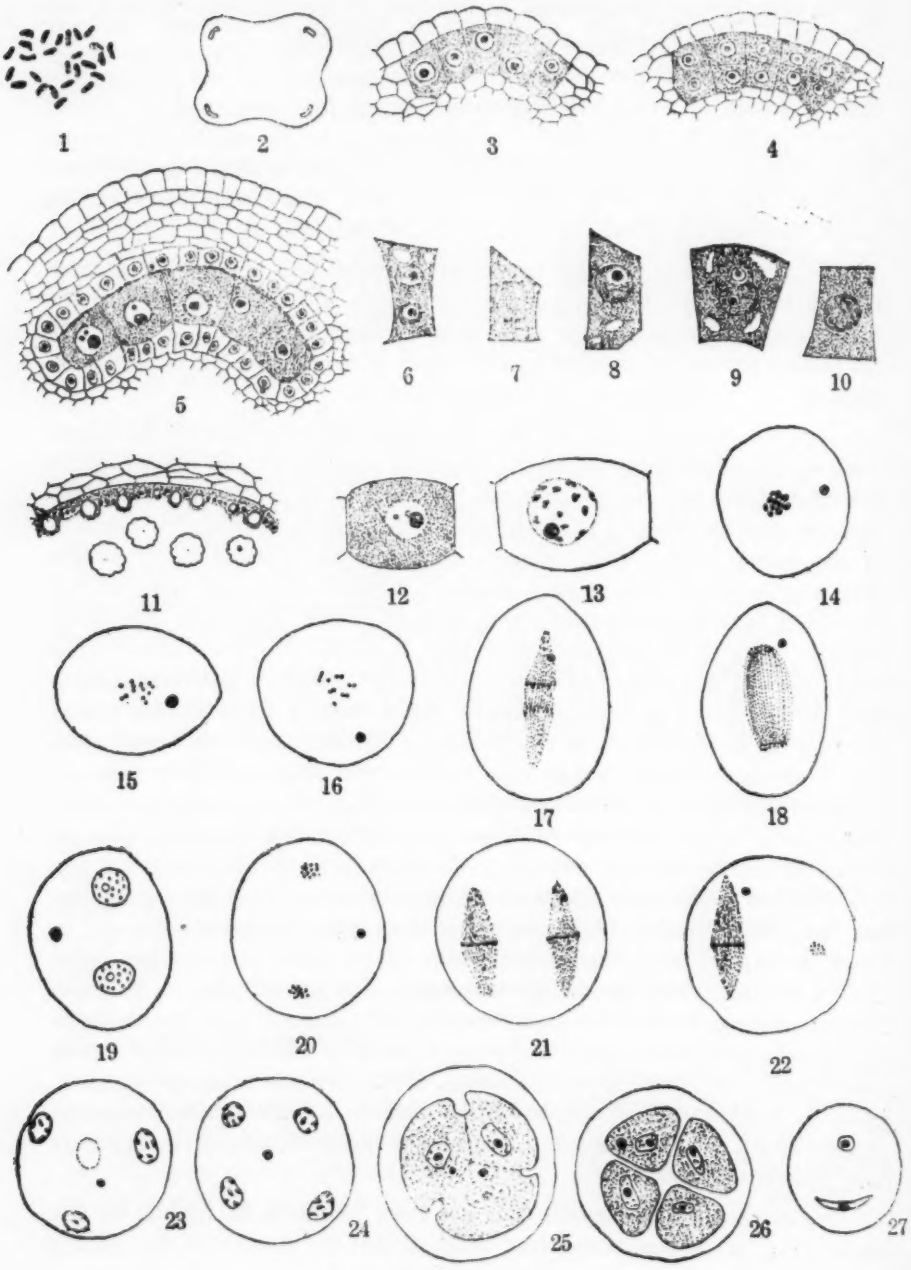
of the chromosome complement on the basis of their morphology was not therefore considered useful and hence not attempted.

Microsporangial development.—The archesporium of the anther consists of groups of 5 or 6 hypodermal cells in the four corners of the anther as seen in Fig. 2. Multicellular archesporia are by no means uncommon. In the closely related genus (*Pedalium*, such extensive archesporia have been recorded (Srinivasan, A. R., 1942). They have also been found in the genus *Nicotiana* (Raghavan and Srinivasan, A. R., 1941 *a*).

To begin with, the single row of archesporial cells (Fig. 3) divide periclinally resulting in the formation of two layers of cells, outer forming the primary parietal cells and the inner primary sporogenous cells (Fig. 4). In the mature anther, the parietal tissue consists of four or five layers, the innermost of which functions as the tapetum (Fig. 5).

It is interesting to note, in this connection, the behaviour of the tapetal nucleus. To start with, the tapetal cells are uninucleate and contain dense cytoplasm (Fig. 6). At a later stage, the nucleus of the tapetal cell shows signs of division resulting in the formation of two nuclei (Fig. 7). Division of the tapetal nucleus takes place even before the nucleus of the pollen-mother cells enters into the early stages of meiosis. After the division, two nuclei are formed within the same tapetal cell, which, after some time, come together. Fig. 8 shows two nuclei after division. In most cases, it has been observed that these two nuclei of the tapetal cells do not remain separate, but that they tend towards fusion. Fig. 9 shows a big binucleate tapetal cell showing the fusion of the two nuclei. There are stages when more than two nuclei are formed by repeated division and ultimately fuse with one another resulting in a tapetal cell with the nucleoli of all the nuclei so fused. Thus in Fig. 10 we find that there are 5 nucleoli within the single nucleus, indicating thereby that the nucleus of the tapetal cell has divided giving rise to 5 daughter nuclei all of which have fused together to form the five-nucleolated nucleus. Cooper (1933) recognises three types of tapetal cells: (1) in which the tapetal cells remain uninucleate, (2) in which they are binucleate and (3) in which they become plurinucleate. The present case in *Sesamum orientale* belongs to the third type described by Cooper. Such plurinucleate tapetal cells have been a common feature in many of the angiospermic genera investigated. In *Gynadropsis* (Raghavan, 1938), in *Chenopodium* (Bhargava, 1936), in *Portulaca* (Raghavan and Srinivasan, A. R., 1941*b*), in *Astercantha* (Rangaswamy, K., 1941) and in *Crescentia* (Venkatasubban, 1944) they have been observed to occur.

The division of the tapetal cells has been found to be mitotic in this species (Fig. 7). It was at one time believed that the division of the tapetals



FIGS. 1–27

TEXT-FIGS. 1–27. *Meiosis in Sesamum orientale Linn.*—Fig. 1. Somatic complement of *Sesamum orientale* showing 26 chromosomes. Fig. 2. Section of a very young anther showing the hypodermal band of archesporium. ×150. Fig. 3. A band of primary archesporial cells. ×150. Fig. 4. Division of the archesporial cells × primary wall cells and primary sporogenous layer. ×150. Fig. 5. Mature anther showing five wall layers, the innermost forming the tapetum. ×150. Figs. 6–10. Tapetal cells showing stages of nuclear division and fusion. ×400. Fig. 6. Binucleate tapetal cell. Fig. 7. Mitotic division of the nucleus. Fig. 8. Uninucleate tapetum. Fig. 9. Fusion of the two nuclei in a single cell. Fig. 10. Cell showing 5 nucleoli within a single nucleus. Fig. 11. Degeneration of the tapetal layer at the pollen grain stage. Fig. 12. Resting nucleus of pollen mother cells showing the budding off of spherical bodies. ×1000. Fig. 13. Diakinesis showing 13 bivalents. ×1000. Fig. 14. Prometaphase. The persistent nucleolus is towards the one side of the cell. ×3,000. Figs. 15 and 16. Metaphase groups showing 13 bivalents in secondary association 1_3, 4_2 and 2_1. The persistent nucleolus is away from the equatorial region. ×3,000. Figs. 17 and 18. Anaphase separation with the persistent nucleolus going ahead of the chromosomes. Fig. 19. Interphase nuclei with the persistent nucleolus towards one side. Fig. 20. Metaphase II showing 13 chromosomes and the separately lying persistent nucleolus. Fig. 21. Spindles of Anaphase II stage lying parallel. Fig. 22. Spindles of Anaphase II stage lying at right angles. Fig. 23. Pollen mother cell showing 3 of telophase nuclei lying in one focus and the fourth in another. Fig. 24. Pollen mother cell showing 4 telophase nuclei all lying in one plane. The persistent nucleolus is in the middle. Fig. 25. Tetrahedral type of tetrad on the process of furrowing. The persistent nucleolus is included in one of the tetra cells. Fig. 26. Isobilateral tetrad cells arranged in one plane. Fig. 27. Two-celled pollen grain at the time of shedding. All figures have been drawn at a magnification of *Ca.* 3,000, unless otherwise stated.

nucleus is amitotic Rocen (1927), in *Portulaca*, and O'Neill (1920), in *Datura*. But that it is through ordinary mitosis has been observed critically and confirmed by Raghavan (1938) in connection with his investigations on *Gynandropsis*. In several other genera investigated in this laboratory, mitosis was found to be the rule. It would thus appear safe to generalise that tapetal nuclear division is through mitosis.

Meiosis.—The microsporangial tissue consists of a single row of five or six microspore mother cells (Fig. 5).

In the resting condition of the nucleus of some of the pollen mother cells, in addition to the big darkly stained nucleolus, small bodies similarly stained but smaller than the nucleolus have been found to occur (Fig. 12). In one and the same loculus of the anther, some pollen mother cells show these bodies while in others they are conspicuous by their absence. Similar bodies have been recorded in the pollen mother cells of various genera, *viz.*, in *Oryza* (Nandi, 1937), in *Hibiscus mutabilis* (Majumdar and Datta, *et al.*, 1934), in *Cicer arietinum* (Iyengar, N. K., 1939) and in *Oenothera rubrinervis* (Gates, 1908). Most of them regard these spherical bodies as extrusions from the nucleoli and consider them to be intermediate stages during the transference of chromatin material from the nucleoli to the chromosomes.

These bodies persist throughout the stages of meiosis right up to the tetrad stage and they have been observed to be included in one of the tetrad cells (Fig. 26).

The possibility of these bodies being chromosomes or their fragments is ruled out for the following reasons, namely, (1) they do not take up any particular position with respect to the cell and are apparently not attracted by forces of attraction or repulsion which are presumed to be responsible for the chromosome movements observed during nuclear division; (2) they are perfectly spherical in shape and homogeneous in structure; (3) they do not undergo any change in their shape or size during meiosis; (4) they are larger than the bivalents or chromosomes at any stage during meiosis, though they are smaller than the prophase nucleolus. That these bodies are nucleolar in origin has been confirmed by positive evidence also. The spherical bodies get stained to the same extent as the nucleolus itself. These bodies seem to bud off from the big prophase nucleolus (Fig. 12) and at various stages, the connection of these bodies with the big nucleolus has been observed clearly. Kumar and Abraham (1942) on their observation in *Sesamum*, call these bodies secondary nucleoli, a name suggestive of their origin.

The behaviour of these spherical bodies during meiosis is indicated at the different stages thereof. In some cases, these bodies were not to be found in the tetrad cells. It is believed that they disappear in the cytoplasm of the pollen mother cells when the tetrads are forming. Probably in most cases they disappear from the scene failing which they are included in one of the tetrad cells.

At diakinesis, the 26 chromosomes of the somatic complement are seen to form 13 bivalents (Fig. 13). These 13 bivalents are mostly of the rod type. They are distributed on the periphery of the nucleus. All the pairs are dispersed at equal distance from each other. This equidistant spacing of the bivalents, according to Lawrence (1931), is due to a repulsion phase which begins at early diakinesis and continues till mid-diakinesis.

The converging movement of the bivalents begins at mid-diakinesis and continues until the bivalents are in close association in the centre of the nucleus. The main nucleolus disappears though the nucleolar bit persists in the form of a spherical body, a little away from the clumped mass of bivalents (Fig. 14).

First metaphase follows prometaphase. The 13 bivalents are arranged on the equatorial plate and are evenly distributed unlike in the case of the sterile hybrid where they are scattered. The bivalents exhibit secondary association, frequently resulting in a number of groups. The maximum

association observed is 1_3, 4_2 and 2_1, thus bringing the total number of groups to 7. Probably this would suggest that the original basic number of the genus is 7. Based on this suggested basic number, the possible origin of the cultivated species has been discussed at the end of the paper. Figs. 15 and 16 show the metaphase plate exhibiting the phenomenon of secondary association. It may be noticed that the persistent nucleolus now takes up a position away from the dividing bivalents. This would naturally indicate that it does not get itself involved in the division of the bivalents and this rules out the possibility of its being chromosomal in nature.

After the metaphase stage, the chromosomes are subjected to anaphasic separation. Anaphase, in this case, is quite normal and the chromosomes disjoin with marked uniformity. The persistent nucleolar body, which is lying away from the equatorial plate during metaphase, is now to be seen at one of the poles. Presumably it has already gone ahead of the chromosomes towards the poles. Figs. 17 and 18 represent the normal anaphase separation and the persistent nucleolus lying at one of the poles.

At each pole, after anaphase, the chromosomes arrange themselves in groups and organize themselves into the interphase nuclei (Fig. 19). Now the nucleolus makes its appearance at both of the interphase nuclei. The chromosomes are more or less uniformly spaced. Such uniform spacing of the chromosomes in the first telophase nucleus has been recorded in *Angelonia* (Raghavan and Srinivasan, V. K., 1940), in *Oenothera* (Gates, 1909) and in *Gynandropsis* (Raghavan, 1938). Gates attributed the uniform spacing of the chromosomes at interkinesis to a mutual repulsion, and the clumping at early telophase, due to attraction. But the "medium in which bodies float frequently change their qualities of attraction and repulsion and it appears that the repulsion first develops after the appearance of the karyolymph in which the chromosomes float". No partition wall is formed between the daughter nuclei nor is the resting stage reached by the interkinesis nuclei (Fig. 19). The persistent nucleolus in some of the cells at this stage occupies a place towards the side of the pollen mother cell while in some cases they were found near one of the telophase groups (Fig. 19).

When second metaphase sets in, the interphase nuclei at either pole lose their nuclear membrane and their nucleoli. The persistent nucleolar body is not involved in it (Fig. 20). At this stage the 13 haploid chromosomes are seen arranged uniformly at the poles.

The second metaphase chromosomes undergo normal disjunction and they reach the poles without exhibiting any irregular phenomenon like bridge formation or fragmentation. Figs. 21 and 22 show the second ana-

phase stage and the persistent nucleolus may be seen on the spindle fibres of one of the anaphase sets. Here also there is an indication by its mere position at the poles that it precedes the chromosomes. The possible causes for the migration of the nucleolus to the poles ahead of the chromosomes have been discussed further below.

The organisation of the spindle during anaphase separation may take place in two ways. In some cells, the spindles lie parallel to each other as in Fig. 21. In others, they lie at right angles to each other so that in one focus, one anaphase group will show the side view of the chromosomes while the other will show the polar view as in Fig. 22. The nature of the tetrads will obviously depend upon the position of the spindles during anaphase. If the spindles are parallel then the four telophase nuclei lie in the same plane (Fig. 24) leading to the formation of iso-bilateral tetrads (Fig. 26). If they are at right angles as in Fig. 22 and 23 then the arrangement is tetrahedral (Fig. 25). Both the types of tetrad arrangement have been noticed in *Sesamum orientale*. The persistent body at this stage is found to be included in one of the tetrad cells (Figs. 25 and 26). Simultaneous furrowing takes place during the formation of the tetrad from the periphery towards the centre. Fig. 25 shows a stage in the process of furrowing. Due to the simultaneous furrowing all the four tetrads are organised simultaneously.

The pollen grain at the shedding stage shows a crescent-shaped generative cell and a small tube cell (Fig. 27). The pollen grains are uniform in size and their wall shows ridges and furrows. All the grains are viable and germinate rapidly in sugar agar cultures. Plate I, Fig. 1, shows a microphotograph of the pollen grains of *Sesamum orientale*.

(*b*) *Sesamum prostratum* Retz

The somatic complement is made up of 32 chromosomes (Fig. 28). They are uniform showing no disparity in size or morphology. All the chromosomes of the somatic complement show terminal constriction.

The microsporangial development and the general outline of meiosis conform to the details already described for *Sesamum orientale*. The tapetal nucleus and its behaviour is also similar to that of *Sesamum orientale*. Fig. 29 shows three tapetal cells in the process of division. In the first cell, anaphase has just set in. In the second cell, the chromosomes have separated and are reaching the poles. In the third cell, two nuclei have ready formed. These figures confirm the mitotic nature of the division of the tapetal cell.

Almost all the pollen mother cells, in their resting condition, show the peculiar phenomenon of nucleolar budding (Figs. 30 *a* to *f*). The nucleolar

buds so formed vary in number and it is found that within a pollen mother cell, in some cases, as many as 7 buds were seen (Fig. 30*f*). But these buds have not been found to persist as in the previous species through meiosis.

Further meiotic stages are normal. Fig. 31 shows first metaphase plate showing 16 bivalents.

(*c*) *Sterile Hybrid*

The diploid complement of the F_1 hybrid shows 29 chromosomes (Fig. 32). Somatic cells of the root tips and of flower buds were examined for purposes of confirmation. Of the 29 chromosomes 16 are derived from the *prostratum* parent and 13 from the *orientale* parent. Since the parental complements showed no morphological disparity among themselves no morphological distinction between these two sets of chromosomes could be recognised in the hydrid complement.

Meiosis: The origin and development of the microsporangium, tapetal behaviour, etc., present no deviation worth any special mention. There is also the same nucleolar budding which was a characteristic feature of both the parents. As many as seven bodies could be seen in the P.M.C. of the resting stage. It is, however, worthy of note that these bodies persist no further. In this respect the hybrid seems to resemble the *prostratum* parent for, in *orientale*, these bodies persist right up to the end. It seems probable that persistence of the nucleolus is a Mendelian recessive. The hybrid shows in some characters, resemblance to the *prostratum* parent a Mendelian dominance. Non-persistence would appear to be dominant to persistence. Hence we find the hybrid showing non-persistence. Full details regarding inheritance of characters by the hybrid are given in a separate paper.

During diakinesis only a few chromosomes pair while the others remain as univalents. The bivalents and the univalents are arranged peripherally around the nucleolus (Fig. 33). The most frequent number of bivalents met with based on an examination of a large number of pollen mother cells is eight.

After diakinesis, the prophase stage sets in when the bivalents and univalents appear clumped at the centre of the cell (Fig. 34). The nuclear membrane disappears at this stage and along with it the nucleolus. This stage comes to an end when the spindle fibres make their appearance.

During Metaphase I the chromosomes separate and unlike in normal meiosis, the chromosomes fail to arrange on the equatorial plate. The bivalents and the univalents are scattered on the spindle. The most frequent arrangement is for the bivalents to occur at the equator and for

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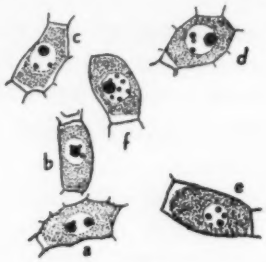

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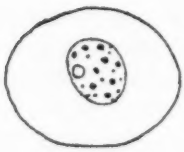

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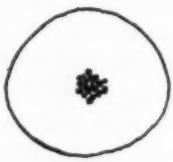

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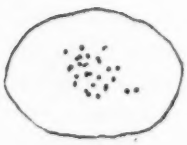

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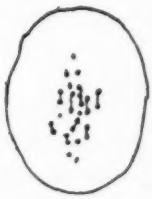

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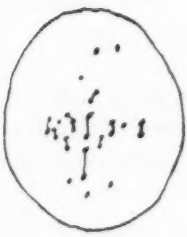

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FIGS. 28–47

TEXT-FIGS. 28–47.—Figs. 28–31. *Meiosis in Sesamum prostratum.* Fig. 28. Somatic plate of *Sesamum prostratum* showing 32 chromosomes. Fig. 29. Three tapetal cells showing mitotic division. ×400. Fig. 30. Resting nucleus of the P.M.C. showing nucleolar budding in various degrees. ×400. Fig. 31. Metaphase I polar view showing 16 bivalents. Figs. 32–47. *Meiosis in the sterile hybrid.* Fig. 32. Somatic complement showing 29 chromosomes. Fig. 33. Diakinesis showing bivalents and univalents. Fig. 34. Prometaphase showing clumped chromosomes. Figs. 35–38. Metaphase I showing bivalents and the univalents scattered. The bivalents are at the equatorial region while the univalents are nearer the poles. Fig. 39. Metaphase I showing 8 bivalents, 1 trivalent and 10 univalents. Fig. 40. Metaphase I showing 9 bivalents, 1 trivalent and the rest univalents. Fig. 41. Anaphase I. Irregular disjunction of the chromosomes. Figs. 42 and 43. Univalents and bivalents lagging in the spindle during anaphase. Fig. 44. Formation of chromosome bridges during anaphase I. Fig. 45. Interphase nuclei showing two cells having unequal number of chromosomes with the left out univalents in the cytoplasm of the P.M.C. Fig. 46. Metaphase II with unequal number of chromosomes. Fig. 47. Anaphase II with the usual lagging chromosomes. All figures have been drawn at a magnification of *Ca.* 3,000, unless otherwise stated.

the univalents to be scattered at the poles (Figs. 35, 36, 37 and 38). Fig. 39 shows 8 bivalents, 1 trivalent and 10 univalents. Fig. 40 shows the metaphase side-view representing 9 bivalents, 1 trivalent and the rest univalents.

There seems to be some relationship between the degree of synapsis and the arrangement of chromosomes in the equatorial region. In all cases where weak pairing is exhibited by the chromosomes this scattered condition prevails. Many cases of haploidy have been cited to show that asynapsis and absence of a regular equatorial plate at Metaphase I, go together. Haplonts of *Nicotiana Tabacum* (Chipman and Goodspeed, 1927) and *Nicotiana glutinosa* (Goodspeed and Avery, 1929) were observed to show this feature. Catcheside (1932) recorded such a behaviour in a haploid *Oenothera* and states in that connection that " many of the chromosomes have never been at the equator of the spindle, but have a definite bias towards one or the other end of the poles ever since diakinesis ". Humphry (1934) has reported such cases in haploid tomatoes. Many examples of interspecific hybrids in the genus *Nicotiana* may be cited to show this prevailing condition of scattered arrangement of the chromosomes, *Nicotiana sylvestris* × *Nicotiana tomentosa* (Goodspeed and Clausen, 1928), *Nicotiana bigelovii* × *Nicotiana solanifolia* and *Nicotiana Tabacum* × *Nicotiana rustica* (Goodspeed, 1934), *Nicotiana glutinosa* × *Nicotiana Tabacum* (Raghavan and Srinivasan, A. R., 1941 *a*).

Thus during first metaphase stage the chromosomes are scattered along the whole length of the pollen mother cell. Their weak pairing during diakinesis and the consequent scattered arrangement of the chromosomes during metaphase constitute cytological basis for the sterility of the hybrid.

The metaphase stage which is characterised by random distribution of the bivalents and the univalents is followed by anaphase which is equally irregular. In normal pollen mother cell, anaphase is characterised by uniform disjunction of the bivalents which results in an equal distribution of chromosomes. But in the case of the hybrid, the bivalents and the univalents during their disjunction exhibit various irregularities.

Fig. 41 shows the migration of the bivalents and the univalents to the poles. Some univalents are seen left out of the spindle and they seem to divide. These divided bits of univalents either reach the poles along with the separating bivalents or they are left out in the cytoplasm where they remain to the last without being included in any of the daughter nuclei.

Frequently bivalents and univalents are seen to lag on a spindle (Figs. 42 and 43). These laggards also get included in one of the daughter nuclei or they remain in the cytoplasm during the interphase stage. These organize themselves into groups and finally form a membrane around them to form the micronuclei (Fig. 50). Sometimes they are found in the plasma in the succeeding stages. In some cases they are included in one of the daughter nuclei. Similar cases of laggards have been recorded in many hybrids. In *Nicotiana* hybrids, *N. glutinosa* × *N. Tabacum* (Raghavan and Srinivasan, A. R., 1941 *a*), in *Brassica* hybrids (Morinaga, 1929) (Ramanujam, 1943), laggards of a similar kind have been found frequently.

Sometimes due to unequal disjunction, the separating chromosomes are connected by long chromatin thread, forming chromatin bridges (Fig. 44). The exact nature of these bridges and the reason for their formation could not be studied in detail.

After the complete separation of the chromosomes, interphase sets in. The two chromosome groups organise into two nuclei at the poles. It is observed that one of the poles contains a larger number of chromosomes than the opposite pole (Fig. 45). This is due to the unequal separation that takes place during anaphase. Further some of the chromosomes have been left out as laggards in the plasma itself. Hence the disparity in number of chromosomes between the two interphase nuclei. No wall is formed between them. Wall formation after the first division is not a common feature of the dicotyledons. But in *Nicotiana* hybrids, between *Nicotiana glutinosa* and *Nicotiana Tabacum* (Raghavan and Srinivasan, A. R., 1941 *a*), wall formation has been recorded.

Second metaphase plate shows two groups of chromosomes distributed with unequal numbers. Some of the laggards are also seen in the cytoplasm. These laggards remain as such and are not included in the second metaphase plate (Fig. 46).

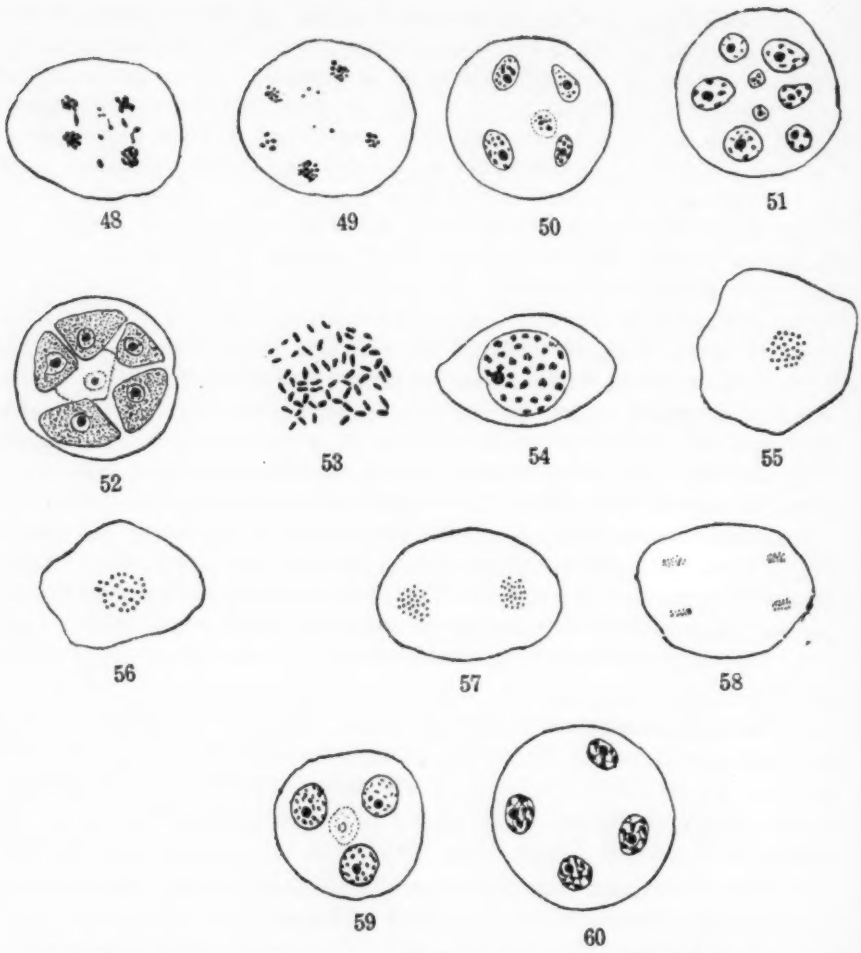
During the succeeding second Anaphase also the chromosomes separate exhibiting irregularities of the kind occurring during first anaphase (Fig. 47). Some chromosomes are left behind on the spindle. Some remain out of the spindle and assemble together. As in first anaphase equal number of chromosomes do not go to each pole. As a result of these, groups having varying numbers of chromosomes are formed and the four daughter nuclei are organised around each one of these groups (Figs. 48 and 49). A few laggards are also seen in the cytoplasm which are not included in any of the telophase nuclei. The discrepancy in the number of chromosomes in the telophase groups is responsible for the formation of the daughter cells of unequal size which ultimately results in tetrads and pentads, big and small. Fig. 51 shows daughter cells of unequal size being formed. Since the chromosomes of the haploid complement are not evenly distributed among the four telophase nuclei, the grains that result from them are non-viable, leading to the sterility of the hybrid. In some cases, some of the laggards that were left out of the spindle arrange themselves in small groups and form micronuclei (Fig. 50). Thus instead of regular tetrads being formed, pentads and hexads result out of the irregularities of meiosis in the hybrids (Figs. 51 and 52). Hence grains exhibit various sizes and shapes resulting in their polymorphic nature. Plate XII, Fig. 3 is a microphotograph of the pollen grains of the sterile hybrid, to show the polymorphic grains. Only 5 to 10% of the pollen grains reached the size of the pollen grains of the parents.

The pollen grains are highly non-viable. They do not germinate even in sugar agar cultures. The pollen grains were deposited on the stigma of both the parents, *Sesamum orientale* and *Sesamum prostratum*. In both the cases, the grains did not germinate. Pollen grains were deposited on the stigma of the sterile hybrid itself. Even then the result proved negative.

Thus it is evident from the above observations that the sterility of the hybrid is the outcome of the irregularity of meiosis.

(*d*) *The Amphidiploid*

Weak pairing between the parental chromosomes in the hybrid indicates that there is no homology between the chromosomes of *Sesamum orientale* and *Sesamum prostratum*. Presumably the parental chromosome sets exhibit structural disparity, hence there is little possibility of their coming together in pairs. This absence of pairing due to the weak homology between the chromosomes could be overcome by supplying these two sets of chromosome complements, each with another homologous set. Thus 13 *orientale* chromosomes may be supplied with another 13 of its own so that both the



TEXT-FIGS. 48–60.—Figs. 48–52. *Meiosis of the sterile hybrid.* Figs. 48 and 49. Anaphase groups of chromosomes containing varied number of chromosomes and also a few left out laggards. Fig. 50. Formation of 4 telophase nuclei and organisation of the micronucleus by the lagging chromosomes. Fig. 51. P.M.C. containing 8 telophase nuclei of different sizes. Fig. 52. P.M.C. containing 6 daughter cells as a result of irregular meiosis. Figs. 53–60. *Meiosis in the Fertile Hybrid* (the amphidiploid) : Fig. 53. Somatic complement showing 58 chromosomes. Fig. 54. Diakinesis showing 29 bivalents. Figs. 55 and 56. Metaphase I showing 29 bivalents at the equator. Fig. 57. Metaphase II showing the equatorial plates containing 29 chromosomes each. Fig. 58. Anaphase II showing normal disjunction. Fig. 59. Telophase nuclei arranged three in one plane and one below. Fig. 60. Telophase nuclei, all the four in one plane. All figures have been drawn at a magnification of *Ca.* 3,000 unless otherwise stated.

sets may pair among themselves. Similarly for *Sesamum prostratum* another set of 16 chromosomes may be made available so that pairing may take place between the two sets of 16 chromosomes. This has been made possible recently by colchicine application by which doubling of genes is brought about.

In the present investigation the 29 chromosomes of the sterile hybrid have been doubled ($2n$: 58). That is, the 13 chromosomes of *orientale* have been duplicated as also the 16 chromosomes of *prostratum*. The result is, the hybrid proves to be completely fertile unlike its sterile predecessor. The cytological explanation of the fertility is the regularity with which meiosis takes place.

Fig. 53 shows a somatic plate containing 58 chromosomes which is double the number of the sterile hybrid ($2n$: 29). But a distinction could not be made between the two parental sets of chromosomes since they were identical in their morphology.

Meiosis.—Microsporangial development is of the normal type as described for *Sesamum orientale.*

Meiosis is regular. At diakinensis there is regular pairing and 29 bivalents are formed (Fig. 54). Metaphase I (Figs. 55 and 56) shows the 29 bivalents arranged in the form of a flat plate in the equator. Obviously pairing has taken place among the duplicated parental genomes, that is, 16 *prostratum* with 16 *prostratum* and 13 *orientale* with 13 *orientale* chromosomes.

First Anaphase is normal and the chromosomes disjoin without exhibiting any irregularity. Fig. 57 shows the metaphase plates during Metaphase II. Second anaphase also is regular (Fig. 58). Equal numbers of chromosomes go to the respective poles. As a result of regular meiosis, the four telophase nuclei are organised with equal number of chromosomes and tetrads are organised in the normal way (Figs. 59 and 60).

The pollen grains that are formed are of uniform shape and size without showing any polymorphism. They are however bigger in size than those of the parents. Plate XII, Fig. 4, is a microphotograph of the pollen grains of the fertile hybrid.

The pollen grains are highly viable as evidenced by the formation of a large number of fruits in the fertile hybrid. Also experiments on germination of the pollen grains in agar culture have shown the rapidity with which the grains germinate. Consequently the fertility of the hybrid has increased the yield of an individual plant by 6 times its parent, *Sesamum orientale,*

The ovary is fertile as revealed by the presence of a large number of seeds which in each fruit amounts to about 50. The amphidiploid thus derived is breeding true and the meiosis in all the subsequent generations have been found to be quite regular. This fertile hybrid may be regarded as a stable true-breeding species, deserving an independent position along with the parents, *Sesamum orientale* and *Sesamum prostratum*.

IV. Discussion

(*a*) *Nucleolus—its behaviour and persistence*

In angiosperms normally the nucleolus appears at the telophase stage and remains till the onset of metaphase, after which it disappears along with the disappearance of the nuclear membrane. However, cases where the nucleolus persists even after the metaphase stage are not uncommon. In *Polanisia trachysperma* persistence upto metaphase in somatic mitosis was recorded by Raghavan (1938). But the nucleolar persistence throughout meiotic stages such as was seen in *Sesamum orientale* is comparatively rare. The behaviour of these nucleolar bodies is varied.

The persistent nucleolus arises as a spherical bud from the nucleolus of the resting nucleus. Many such buds are formed, most of them disappearing during diakinesis stage except one which persists with the same size neither diminishing nor dividing until finally incorporated in the tetrad cells. There are also cases where this body after remaining for most of the stages of meiosis, disappears into the cytoplasm.

The possibility of these bodies being chromosomes or their fragments is ruled out by the fact that they are bigger than the chromosomes and perfectly spherical in shape. They take the stain to the same extent as the nucleolus does. During meiotic stages they do not play any part. Thus it may be seen that by their origin from the margin of the big nucleolus and by their taking up the stain to the same extent, these bodies are nucleolar in nature.

The persistent nucleolus in the pollen mother cells of *Sesamum orientale* is observed to exhibit varied movements. During the earlier stages of meiosis it either remains in the equator or lies apart. If it remains in the equator it migrates to the pole when anaphase separation sets in; or in some cases it disappears altogether. If they persist they get incorporated into one of the tetrad cells. Thus it stands to reason that some force has been acting upon these persistent nucleolar bodies to enable them to execute such movements.

The factors which are responsible for such a movement have not been clearly established. They are presumably acted upon by the same forces

which are responsible for chromosome movements, like the contraction of spindle fibres, electromagnetic repulsion or attraction set up by cytoplasmic currents in the spindle region. Mensinkai (1939) regards the division and migration of the persistent nucleolus as being due to the stretching of these spindle fibres. But since the persistent nucleolus has been found to have no connection with the spindle fibre, it is unlikely that the contraction of the spindle brings about the movement of the nucleolus. The other alternative is the magnetic attraction set up by the cytoplasmic current. The fact that sometimes the persistent nucleolus remains at the equator while at other times it moves towards the poles would suggest that these movements may be due to the above cause. Further the spindle region would appear to be one of localised forces and when a body lies in that region it is carried away provided it is not attached to any thing like the spindle fibre. The persistent nucleolus by its position at the border of the spindle fibres and also by its migration to the poles ahead of the chromosomes, it would appear that the movement of the nucleolus might be controlled by the localised forces that have been referred to above.

During metaphase the chromosomes are attached to the spindle fibres and since contraction of the fibres takes place only a little later, the chromosomes are prevented from being carried away by the forces at the spindle area, while the nucleolus lies unattached to the spindle fibres so that it is free to move and hence it is found to reach the poles ahead of the chromosomes. Movements of this kind may perhaps be explained by the electromagnetic theory of nuclear division (Kuwada and Sugimoto, 1928). According to this theory, the persistent nucleolus, because of the presence of plastin, which it has retained instead of giving to the chromatin, becomes highly electropositive, while the poles remain oppositely charged. As a result of this the persistent nucleolus is attracted towards the pole and hence the movement. It is further explained that in the normal cases where there is no persistent nucleolus, the chromosomes change their electric charge due to the transference of the plastin from the nucleolus. A similar explanation for the differential movement of the persistent nucleoli and the chromosomes would seem probable in the present instance also.

(*b*) *Interspecific hybridisation—a guide to ancestral homology.*

Interspecific and intergeneric hybridisations and the study of the behaviour of such hybrids have long been engaging the attention of many cytologists, since the results of the observation serve as valuable clues to determine the relationship between the various species. The mode of origin of new species can be inferred with the help of hybridisation results as disclosed by cytological data.

Species having the same number of chromosomes when crossed with each other will give either fertile hybrids or hybrids of partial or completely sterile nature. Such fertile hybrids are met with in the following cases. *Viola* (Clausen, 1931), *Nicotiana* (Goodspeed, 1934) and *Triticum* (Aase, 1930). Clausen (1931) crossed *Viola tricolour* (n: 13) with *Viola alpestris* (n: 13) and got a hybrid which was fertile ($2n$: 26). During meiosis, he observed that 13 chromosomes of *Viola alpestris* paired completely with 13 chromosomes of *Viola tricolour* thus resulting in a fertile hybrid. The complete synapsis in this case indicates the complete homology of the two sets of chromosomes. Hence these two species, though taxonomically distinct may be regarded as having had a common origin on the basis of this piece of cytological evidence.

In the case of sterile hybrids, some show partial pairing with varying number of bivalents and univalents during meiosis. In such cases the greater the number of bivalents formed, the greater has been the fertility of the hybrids. For instance, Clausen (1931) crossed *Viola nana* (n: 24) with *Viola lutea* (n: 24). The hybrid was found to be partially fertile; meiotic stages showed the presence of only a few bivalents 6 to 8. In the normal case if there is complete pairing there should be 24 bivalents formed. But as there were only a few bivalents, the hybrid was partially sterile; correspondingly in the hybrids between *Viola orphinidis* (n: 11) and *Viola cornuta alba* (n: 11) he found a greater number of bivalents amounting to 9 or 10. The hybrid was almost completely fertile. Thus it would appear that the degree of hybrid fertility is directly proportional to the number of bivalents formed during the meiotic stages of the hybrids.

In the case of some hybrids, pairing is totally absent and consequently the hybrids are completely sterile. Karpechenko (1927 *a*, 1927 *b*) got a hybrid which was completely sterile, by an intergeneric cross between *Raphanus sativus* (n: 9) and *Brassica oleracea* (n: 9). This complete sterility was attributed to the total absence of synapsis or pairing of parental chromosomes during meiosis. This only confirms the previous inference that the degree of synapsis is a measure of the degree of hybrid fertility.

Thus it is noticeable that though the two parental chromosomes of hybrids are equal in number, yet they vary in their degree of affinity indicating thereby that the pairing of chrmosomes does not depend upon the numerical identity of the chromosomes but on their structural and morphological homology. This homology between chromosomes of two gametic sets will be nearer if both the parents have had a common origin. Thus the cytological behaviour of species hybrid indicates not only the extent of homology between the species but also the ancestry of the parental forms.

In the case of hybrids derived out of parents having different chromosome numbers, the behaviour of hybrids exhibits complication which is nonetheless interesting.

The behaviour and the extent of affinity of the chromosomes in such hybrids as disclosed by their behaviour at meiosis has been classified by Tackholm (1922) into three groups. They are (1) *Drosera* scheme of pairing where there is strong affinity between parental chromosomes; (2) *Hieracium Boreale* type where there is a weak affinity; and (3) the *Pygrarea* type where there is no affinity. It was Rosenberg (1909) who first observed this phenomenon of pairing in *Drosera* hybrids. He crossed *Drosera rotundifolia* (2*n*: 20) with *Drosera longifolia* (2*n*: 40). As a result he got a hybrid containing 30 chromosomes, 10 from the *rotundifolia* parent and 20 from the *longifolia* parent. During synapsis only 10 bivalents were formed and 10 chromosomes remained as univalents. Rosenberg concluded that the 10 chromosomes of *rotundifolia* paired with 10 of *longifolia*, leaving the other 10 of *longifolia* unpaired. Such a type of pairing between two sets of chromosomes belonging to two different parental species which may or may not have equal number of chromosomes is known as Allosyndesis. Here 10 chromosomes of *rotundifolia* and 10 chromosomes of *longifolia* paired allosyndetically. Similar cases of allosyndesis have been recorded in *Triticum* hybrids (*n*: 35) resulting from a cross between *Triticum Emmer* (*n*: 14) and *Triticum Vulgare* (*n*: 21). 14 synaptic pairs were formed. 14 chromosomes of *Triticum Emmer* paired with 14 of *Triticum Vulgare* while the remaining 7 chromosomes of *Vulgare* parent were left in an unpaired condition (Kihara, 1919; Sax, 1922). In *Nicotiana* hybrids between *Nicotiana Tabacum* (*n*: 12) and *Nicotiana sylvestris* (*n*: 24) (Goodspeed and Clausen, 1927) there was an arrangement of 12 bivalents and 12 univalents indicating allosyndesis between 12 chromosomes of *Nicotiana Tabacum* and 12 chromosomes of *Nicotiana sylvestris* (*n*: 24).

There are also cases where in addition to allosyndesis there is also autosyndesis. Autosyndesis indicates the pairing among the chromosomes of a single set. Thus in *Digitalis* hybrids (2*n*: 72), between *D. lutea* (*n* : 48) and *D. micrantha* (n: 24) 72 chromosomes were found in the somatic complement, and during meiosis, they organised into 36 gemini (Haase-Bessel, 1916) indicating that all the chromosomes have paired. It means that 24 chromosomes of *D. micrantha* have paired with 24 chromosomes of *D. lutea* to form 24 bivalents. The remaining 24 chromosomes of *D. lutea* have paired among themselves to form 12 more bivalents, thus bringing the total to 36 bivalents. In such a case as this, there is not only pairing between the members of the gametic complements of the two different species, namely,

D. lutea and *D. micrantha* (allosyndesis) but also among the remaining chromosomes of the same gametic complement, namely *D. lutea* (autosyndesis).

Similarly in *Papaver* hybrids (Ljundahl, 1924), *viz.*, *Papaver nudicaule* (n: 7) and *Papaver radicatum* (n: 35) there are 21 gemini formed which may be explained on the same basis. 7 chromosomes of *nudicaule* have paired with 7 of *radicatum* and the 28 chromosomes of *radicatum* have paired among themselves to form 14 gemini. Thus there is allosyndesis between 7 of *radicatum* and 7 of *nudicaule* and autosyndesis between the 28 chromosomes of *radicatum* themselves. This revealed that though the two parental complements differ in number yet there is a marked affinity between the two sets. Allosyndesis and Autosyndesis would thus indicate the extent to which there exists homology between the two parents.

In hybrids exhibiting weak pairing among the chromosomes of the parents, varying degrees of synapsis and in some cases asynapsis also occur in the meiotic cycle of the hybrid and consequently it becomes sterile. Raghavan and Srinivasan, A. R. (1941 *a*) record such weak pairing among the chromosomes in the hybrids between *Nicotiana glutinosa* ($2n$: 24) and *Nicotiana Tabacum* ($2n$: 48). They observed varying degrees of synapsis and also in certain cases complete asynapsis. This would indicate distant homology. This hybrid has been classified by them under the *Hieracium Boreale* type. Hybrids belonging to the last scheme, namely, showing no affinity between the members of the two complements have been recorded in many cases. *Crepis* (Collins and Mann, 1923), *Digitalis* (Haase-Bessel, 1921) and *Nicotiana* (Goodspeed, 1934).

In the present investigation the meiosis of the sterile hybrid was studied in detail with a view to find out the degree of affinity that existed between the two sets of gametic complements. The sterile hybrid ($2n$: 29) of the cross between *Sesamum orientale* (n: 13) and *Sesamum prostratum* (n: 16) shows irregular meiosis with varying numbers of bivalents. It is also found that in the majority of cases 8 bivalents are formed with the rest scattered as univalents. The somatic number 29 of the hybrid should contain 13 of *orientale* chromosomes and 16 of the *prostratum* parent. If it conforms to the *Drosera* scheme of pairing, then 13 of *orientale* chromosomes should pair with 13 of *prostratum*, leaving the three *prostratum* chromosomes unpaired. But such a maximum pairing has never been observed to take place as most of the pollen mother cells show 8 and very occasionally 10 bivalents. This can be interpreted in two ways: (1) That the 8 chromosomes of *orientale* pair with a corresponding number of *prostratum* chromosomes leaving the others

unpaired. This means that it is a case of allosyndetic pairing. (2) That the *prostratum* chromosomes might pair among themselves to form 8 bivalents, leaving the 13 *orientale* chromosomes in an unpaired condition. This suggests autosyndesis among *prostratum* chromosomes. Either of these interpretations would indicate only a weak homology between the chromosomes of *Sesamum prostratum* and *Sesamum orientale*. It is therefore reasonable to infer that we have to look to some other source for the origin of the cultivated til (*Sesamum orientale*) than from *prostratum*. It is not likely that they could have had a common origin on account of their distant homology as revealed by the behaviour of their chromosomes in the hybrid. This would appear to be supported also by the fact that even though they belong to the same genus, they are different in their habit. The one is erect whereas the other is prostrate. *Sesamum orientale* is an annual herb whereas *Sesamum prostratum* is perennial almost a shrub. It may be that future explorations into the Indian wilds may show the presence of ancestral forms of the domesticated til. In this connection, we have also to remember the American tropics. Only one wild species has been reported from Argentina, which is *Sesamum radiatum* ($2n$: 64) (John and Rao, 1941). Its number suggests tetraploidy from *Sesamum prostratum*. Whether there are any more forms which could throw light upon the origin of the cultivated til, future exploration alone can reveal.

(*c*) *Artificial synthesis of a new species*

The genes on the chromosomes govern plant characters. Any alteration of the genes either in their position or in their number would consequently affect the configuration of a plant. Gene mutations thus bring about mutations of plant characters. Generally gene mutations involve a rearrangement of the genes such as inversion, reciprocal translocation, etc. These lead to mitotic and meiotic aberrations resulting in external morphological mutations of several kinds. A more common and fruitful way in which changes in plant configurations occur is through the duplication of genes of certain chromosome sets or the duplication of the entire genic complement. That is, all the members of the chromosome complement undergo reduplication and this is known as Polyploidy. The phenomenon of polyploidy may be of two kinds, Autopolyploidy and Allopolyploidy. In the former there is a duplication of the chromosomes derived from the same parent (as in self-pollinated plants) or from parents belonging to the same species as in cross-pollinated plants. In the latter two sets of chromosomes from two different parents are involved. This may happen in interspecific hybrids and very rarely in intergeneric hybrids.

Autopolyploids arise either spontaneously in nature or are artificially induced. Allopolyploidy on the other hand indicates hybridisation, whether interspecific or intergeneric. Autopolyploids may be stable species breeding true. In most cases where there is induced polyploidy sterility of the autopolyploid is quite common as in *Cosmos* (Earl Newcomer, 1941). Allopolyploids as aforesaid arise out of hybridisation. The hybrid so derived may be sterile or fertile. If the hybrids prove fertile, then the allopolyploids breed true and establish themselves as stable species. Allopolyploids which are sterile due to hybridisation may be made fertile by artificial induction of amphidiploidy about which a detailed mention is made further below.

The most common form of autopolyploids occurring in nature are the tetraploids. Tetraploids arise as a result of the duplication of the diploid chromosomal set. Many causes are in evidence for the duplication of chromosomes in the plant cell. Cytomyxis, occurring in the pollen mother cells, is considered by some to be one among them. This phenomenon was first observed by Gates (1911) in the pollen mother cells of *Oenothera gigas*. He described the process as a migration of the chromatic material from the one pollen mother cell into the adjacent cell. But he contended that the chromatic material disappeared into the cytoplasm of the recipient cell and that the chromatic material of the recipient cell was not increased by the addition of extruded chromatic material from the adjacent cell. Thus according to him, cytomyxis does not bring about chromosome duplication. Binucleate pollen mother cells arisen from cytomyxis, have been recorded in *Tridax* (Raghavan and Venkatasubban, 1941) where the two nuclei enter independently into successive division stages and ultimately it was observed that this phenomenon was responsible for the degeneration of the pollen mother cells and the significant sterility in the species was attributed to cytomyxis. Nandi (1937) also describes such cases of binucleate pollen mother cell formation from cytomyxis at diakinesis in *Oryza*. Particularly in the case of hybrids both interspecific and intergeneric this abnormal phenomenon seems to be quite common. Kattermann (1933) in *Triticum* × *Secale* hybrids, Percival (1930) in hybrids between *Aegilops* × *Triticum* species and Raghavan and Srinivasan, A. R. (1941 *a*) in *Nicotiana glutinosa* × *Nicotiana Tabacum* hybrids. From this and from the evidence of Church (1929) who found the occurrence of this phenomenon in the hybrids of *Phalaris*, we may infer that it is more probable that this phenomenon is associated with hybrids than being an artifact as Sinoto (1922) regarded. It cannot however be said with any amount of certainty whether the formation of additional nuclei through cytomyxis is a certain method of origin of polyploidy.

Tetraploidy may also arise from fusion of unreduced gametes having the diploid number of chromosomes. They are formed due to absence of cross-wall formation after the heterotypic division. When an unreduced gamete fertilises a gamete with the haploid number, then a triploid results. If it fertilises another unreduced gamete, a tetraploid is formed. Such instances of tetraploidy are common in *Datura*, and Tobacco. In *Brassica* hybrids it was observed by Ramanujam and Srinivasachar (1943).

Experimental tetraploids have been obtained in a number of species. As early as 1914 Gregory described a tetraploid strain in *Primula sinensis*, containing 48 chromosomes. It has been shown that in the chromosome sets of diploids there are chromosomes of different kinds, each of which is represented twice, one of the two being derived from egg and the other from the pollen. In the tetraploids with 48 chromosomes, it was found that the chromosomes often came together in fours at reduction division. It was found that the 48 chromosomes of the tetraploid united into 12 groups of four. Winkler (1916) induced polyploidy in *Solanum* by grafting together the species *Solanum lycopersicum* and *Solanum nigrum*. The adventitious shoots arose at the grafting point were in some cases tetraploid. Decapitation is another means of bringing about tetraploidy. Terminal buds of tomato, tobacco have been decapitated and callus allowed to form which produced adventitious buds from which arose tetraploids (Beadle, 1940).

Certain drugs, particularly the alkaloid, Colchicine, extracted from *Colchicum autumnale* have been known to produce characteristic disturbances in the cell division. Blakeslee and Avery (1937) showed that treating seeds with an appropriate solution of colchicine produces tetraploid tissues from which tetraploid strains may be derived. Their work has been subsequently confirmed and employed by many investigators (Nebel and Ruttle, 1938), (Levan, 1938, 1939 and 1940 *a*) and many others.

An important property of Autopolyploids concerns the behaviour of their chromosomes at meiosis. In a diploid organism, every chromosome has its homologous partner. Of the two homologous sets one is from the male cell and the other belongs to the female sexual cell. A number of bivalents equal to the haploid chromosome number is formed and the disjunction at meiosis gives rise to gametes all of which contain haploid sets of the chromosomes. In an autopolyploid every chromosome has more than one homologue so that opportunity presents itself for the formation of trivalents, quadrivalents and higher associations. The disjunction at meiosis is frequently abnormal, different numbers of chromosomes going to two poles of the division spindle. In order to breed true, an autotetraploid

must produce gametes all of which have the same complements of chromosomes. Since loss or addition of chromosomes usually reduces the viability of the offspring, the reproductive cells of polyploids are frequently non-functional. But there are a number of cases of tetraploids which are normal and consequently breed true and have established themselves as stable forms, *e.g.*, *Tradescantia* (Anderson and Sax, 1936).

Apart from their existence in nature, due to natural hybridisation, allopolyploids have been produced experimentally. The intergeneric hybrids between radish (*Raphanus sativus*, n: 9) and cabbage (*Brassica oleracea*, n: 9) serve as an illustration of the results obtained when the chromosome complement is reduplicated in crosses of taxonomically remote forms (Karpechenko, 1927 *a* and *b*). Both parents have the diploid number 18. Cross succeeded fairly easily. The hybrids had 18 chromosomes, 9 from the radish and 9 from the cabbage parent. No chromosome pairing took place and the 18 chromosomes remained as univalents at metaphase of the first division and were distributed at random to the poles. At the second division the univalents split, giving rise to cells with a varying number of chromosomes, mostly from 6 to 12. In some of the pollen mother cells however, the first division was abortive and nuclei were formed that included all the 18 univalents. The second division then gave rise to two diploid spores. Two pollen grains containing the diploid complements were organised. The F_1 hybrids mostly were sterile but few seeds were produced. Cytological examination showed that most of the F_2 hybrids derived from the seeds had 36 chromosomes in their somatic cell. The origin of such plants was in all probability due to the union of the few diploid gametes produced in the F_1 hybrid. The F_2 plants therefore contained 18 radish and 18 cabbage chromosomes; in other words, the diploid complement of the chromosomes of each parental species. Such F_2 hybrids were allotetraploids. The meiotic divisions were very regular in striking contrast with the abnormalities observed at meiosis in the F_1 hybrids. In the tetraploids, 18 bivalents were formed, disjunction was normal and the resulting cells contained 18 chromosomes each. It is practically certain that 18 bivalents that appeared at meiosis were due to the pairing of 9 radish chromosomes with their 9 radish homologues. Thus the pairing was between similar chromosomes of the same parent (Autosyndesis) rather than between the chromosomes of different species (Allosyndesis). The tetraploid plants were fertile and bred true. This true breeding type was assigned the name *Raphano-Brassica* because it arose out of the two genera, *Raphanus* and *Brassica*, after hybridisation.

Raphano-Brassica is by no means the only new species which has arisen through allopolyploidy in experimental cultures. *Primula Kewensis* (n: 18

and $2n$: 36) is another allotetraploid which arose as a bud sport among population of *Primula floribunda* and *Primula verticillata*, both having haploid number 9. *Primula Kewensis*, the diploid hybrid of *Primula floribunda* and *Primula verticillata*, was observed to set seed on three occasions since its first production in 1900 (Newton and Pellew, 1929). Each time its seed gave rise to fertile plants with the tetraploid number of chromosomes, $2n$: 36. In the vegetative cells of one of the fertile inflorescences, tetraploid number of chromosomes was found, showing that the doubling process took place in the somatic division. It was the only case known of a sterile (diploid) hybrid giving rise to a fertile tetraploid by somatic doubling of chromosomes. In the meiotic division of the diploid hybrid of *P. Kewensis* (n: 18) 9 pairs of chromosomes were formed which may be indicated as F1 V1, F2 V2 and so on. The resulting gamete would contain all possible combinations of chromosomes. Most of these gametes were non-viable; a few however were viable and these while they bore many *P. floribunda* characters also showed traces of *P. verticillata*. But in the tetraploid hybrid each chromosome was represented twice and if 18 pairs were formed in meiosis, they might either be pairs of identical chromosomes (F1 F1, V1 V1) or of corresponding *floribunda* and *verticillata* chromosomes (interspecific pairing) as in the diploid hybrid. In the last case, the number of possible combinations would be much greater than in the diploid. In the former case, F1 F1, V1 V1 or identical chromosomes separate and the gamete will each contain a complete set of *floribunda* or *verticillata* chromosomes which on fertilisation will give a uniform progeny. Thus the hypothesis of pairing of identical chromosomes (intraspecific) gives a satisfactory explanation of a perfectly constant tetraploid hybrid. This hypothesis was put forward by Winge (1917) in discussing the possible origin of tetraploids from hybrids. He considered that doubling of chromosomes might result in failure to conjugate at meiosis, followed by splitting and subsequent pairing of the identical halves.

The case of *Crepis* is somewhat different from that of *Primula Kewensis*. Poole (1931) showed that in the diploid hybrid of *Crepis*, *Crepis rubra* (n: 5) × *Crepis fœtida* (n: 5) there was complete pairing of the chromosomes. They behaved as though they were from the same parents. Consequently, the hybrid was fertile and the tetraploid derived from it behaved almost like an autotetraploid. Quadrivalent formation was very common. In the F_1 hybrid R (rubra) and F (fœtida) chromosomes paired (RF). In the tetraploid form duplication of the chromosomes took place, resulting in RR and FF. Because of the complete homology of R and F chromosomes, these four chromosomes formed one quadrivalent (RRFF). But in the case of

Primula Kewensis tetraploid (FFVV) there were no quadrivalents formed. Instead F and F paired and V and V paired forming 18 bivalents. It might be that even though F and V were somewhat related, they were not completely homologous so as to induce quadrivalent formation. Presumably VV and FF bivalents may exhibit secondary association indicating their ancestral relationship.

Experimentally-produced allopolyploids of the kind described above happen to be identical to wild Linnæn species already existing in nature. The classical example of such an allotetraploid is that of *Galeopsis Tetrahit*, an existing Linnæan species which was experimentally synthesised from its putative ancestors. In his monograph on the genus *Galeopsis*, Muntzing (1930, 1932 and 1937) showed that six out of the eight species investigated had the haploid number of chromosomes 8 and the two remaining ones had n: 16. Among the former were the species of *Galeopsis pubescens* (n: 8) and *Galeopsis speciosa* (n: 8) and among the latter was *Galeopsis Tetrahit* (n: 16). The crosses between *G. pubescens* and *G. speciosa* succeeded easily when *G. pubescens* was used as the female parent. At meiosis varying numbers of bivalents and univalents were formed. The anther of the flowers of this hybrid contained only 8 to 20% of good pollen grains. A few good ovules however were produced. In the F_2 progeny raised by the few seeds obtained, a single plant was found that proved to be a triploid ($3n$: 24). Its origin is probably due to the union of a gamete containing the somatic complement of the hybrid (8 chromosomes of *G. pubescens* and 8 chromosomes of *G. speciosa*) with a gamete carrying 8 chromosomes. This triploid was backcrossed to pure *G. pubescens*. A single seed resulted from the backcross. It gave rise to a plant which proved to be a tetraploid ($4n$: 32). This tetraploid was fertile and became the progenitor of a strain which was named " artificial Tetrahit".

This ' artificial Tetrahit ' was like the real *Galeopsis Tetrahit* described by Linnæus, in possessing 32 chromosomes in somatic cells and 16 bivalents at meiosis. The irregular meiosis characteristic of the F_1 hybrids ceased to exist in the artificial Tetrahit. In short, the artificial *G. Tetrahit* and the natural species are similar not only in their morphology but also in their genetical and cytological behaviour.

Spartina Townsendii ($2n$: 56) is another example of an experimental allotetraploid. *Spartina stricta* (n: 28) and *Spartina alternifolia* (n: 35) were crossed (Huskins, 1931). The tetraploid form of the hybrid was found to contain 126 chromosomes. *Spartina Townsendii* showed a diploid number of 126 chromosomes and with morphological and cytological evidences,

Huskins proved that *Spartina Townsendii* was an allotetraploid derivative of the hybrid between *Spartina stricta* and *Spartina alternifolia*.

Since the discovery of colchicine as an agent for the doubling of chromosomes, several experiments have been conducted to confirm the origin of existing species by artificially repeating the supposed event that led upto their formation. Thus existing polyploid species have been artificially synthesised from their putative ancestors, in *Nicotiana* (Greenleaf, 1941), in *Gossypium* (Harland, 1940) and in *Triticum* (Thompson, Britten and Harding, 1943). Recently *Brassica juncea* was artificially synthesised and its origin was traced with the help of cytological and cytogenetical evidences (Ramanujam and Srinivasachar, 1943). According to Morinaga (1934) *Brassica juncea* ($2n$: 36) is an allotetraploid composed of the genomes of *Brassica campestris* (n: 10) and *Brassica nigra* (n: 8).

Two evidences were adduced to the allotetraploid origin of *Brassica juncea*. Firstly in a cross between *Brassica juncea* (n: 18) and *Raphanus sativus* (n: 9) there was complete absence of pairing among the *B. juncea* chromosomes themselves in the F_1 hybrid. Secondly, when crosses were made on the one hand between *B. juncea* and *B. campestris* and on the other between *B. juncea* and *B. nigra*, the Drosera scheme of pairing was observed. That is in the F_1 hybrids (*B. juncea* × *B. campestris*) and (*B. juncea* × *B. nigra*) the configuration of 10 bivalents and 8 univalents and 8 bivalents and 10 univalents occurred respectively. In *B. juncea* × *B. campestris* 10 chromosomes of *B. campestris* paired with 10 of *B. juncea*, leaving the 8 chromosomes of *B. juncea* as univalents.

It is clear from the regular formation of bivalents in these hybrids that the haploid set of *B. juncea* chromosomes is equivalent to the haploid set of the two species, *B. nigra* and *B. campestris* and that by doubling the chromosomes of the F_1 hybrid got between them, plants resembling *B. juncea* could be produced.

Recently such an origin of *Brassica juncea* as an allotetraploid from *B. campestris* and *B. nigra* parents has been confirmed by the more direct evidence of synthesising the species by successfully effecting crosses between the two parents and subsequently inducing amphidiploidy by the application of colchicine (Ramanujam and Srinivasachar, 1943). Additional confirmation was obtained from the fact that there was uniform pairing between synthetic *B. juncea* and natural *B. juncea* when they were crossed. They crossed *B. campestris* and *B. nigra* and the F_1 hybrid that resulted out of this cross possessed $2n$: 18 and these appeared as bivalents and univalents during meiosis. Occasional cases of quadrivalent formation were also met

with. Anaphase I and II were characterised by bridge formation. Fruit setting was very poor and only a few seeds could be available. The first generation of Amphidiploid was produced as a chimeral branch on F_1 hybrid of the above cross. Two branches were treated with ·4% colchicine in 50% glycerine. The branches treated showed fertility and an A_2 generation of plants were raised from the seeds available in the fruits of the treated branches. The A_2 generation possessed diploid set of 36 chromosomes and resembled in all respects those belonging to the species *B. juncea*. This amphidiploid crossed easily with the natural *B. juncea*. Pairing was complete thereby indicating that the haploid set of the amphidiploid was homologous to the haploid set of *B. juncea*. Indirect evidences as adduced by Morinaga (1934) stand confirmed by this direct evidence through artificial synthesis.

In a manner similar to the above mentioned cases, hybridisation and artificial induction of amphidiploidy led to the establishment of a true breeding species of *Sesamum* in this laboratory. The amphidiploid, details of whose characters are given in another paper, proved to be a stable true-breeding type and deserves an independent place along with the parental species, namely, *Sesamum orientale* and *Sesamum prostratum*. *Sesamum orientale* ($2n$: 26) and *Sesamum prostratum* ($2n$: 32) were crossed reciprocally. This resulted in a hybrid having $2n$: 29. Meiosis was found to be irregular because neither complete autosyndesis nor complete allosyndesis was observed. The result was that the hybrid proved to be sterile. A duplication of the chromosomes means the duplication of the parental chromosome sets. Then during meiosis there would be autosyndesis which would result in regular meiosis. Thus with this object in view the sterile hybrid was treated with colchicine and amphidiploidy was successfully induced.

Colchicine solution in tap water of strength ·4% was applied in the form of drops at the terminal bud of young seedlings of the hybrid. The treatment was given twice a day on three alternate days. A cotton wool was placed at the region of application to prevent excessive evaporation of the chemical. The colchicine effect was revealed in the hybrid by its stunted growth and deformed leaves. Flowers were formed which were almost twice as big as those of the sterile hybrids. Viable pollen grains were formed and the treated seedlings yielded fruits with good seeds. Thus fertility was induced through amphidiploidy.

The cause of the fertility may be inferred as follows: The 29 chromosomes of the hybrid plant would have been doubled to 58 by the action of colchicine so that the somatic complement instead of having 16 chromosomes

of *prostratum* and 13 chromosomes of *orientale* would have 32 chromosomes of *prostratum* and 26 chromosomes of *orientale*. During meiosis no irregularity was noticed because the 32 chromosomes of *Sesamum prostratum* paired autosyndetically, to form 16 bivalents and the 26 chromosomes of *Sesamum orientale* parent paired autosyndetically among themselves to form 13 bivalents, thus resulting in autosyndesis or intraspecific pairing in both the parents. The 29 bivalents observed in meiosis of the amphidiploid must be the total number of bivalents of both the parents. This amphidiploid has been established as a stable true breeding type evolving out of interspecific hybridisation followed by amphidiploidy.

The true breeding fertile hybrid resembles the *prostratum* parent more than the *orientale* including the perennial habit. Even the sterile hybrid shows greater resemblance to *Sesamum prostratum*. The cytological explanation for this may lie in the fact that both in the sterile and fertile hybrids there is a greater number of *prostratum* chromosomes. The F_1 hybrid is only an annual whereas the amphidiploid is a perennial. Possibly the presence of a very large number of *prostratum* chromosomes, 32 in a complement of 58, is responsible for incorporating this parental feature also in the amphidiploid. Thus cytological investigations of many of the existing species, wild and cultivated, may well show that, in speciation, amphidiploidy has played an important role. Many of the existing forms may be proved to be amphidiploids, provided their parental ancestors are discovered. Thus in evolution of new species, allopolyploidy has played an important part.

(*d*) *The possible origin of the cultivated Til, Sesamum orientale* Linn.

From the cytological data gathered through interspecific hybridisation, it is safe to infer, in an empirical way, the possible origin of the cultivated Til. If, in the interspecific hybrid between *Sesamum orientale* Linn. and *Sesamum prostratum* Retz., there was exhibited Drosera scheme of pairing, then it would have meant that the genome of *Sesamum prostratum* contained within it the genome of *Sesamum orientale* and consequently the two would be related ancestrally. But that is ruled out.

The frequent occurrence of 8 bivalents can be regarded as autosyndetic pairing amongst the 16 *prostratum* chromosomes, leaving the 13 chromosomes of *orientale* unpaired.

Or it may be that the 8 chromosomes of *prostratum* have paired with 8 chromosomes of *orientale* to form the bivalents. In this case 8 chromosomes of *prostratum* and 5 chromosomes of *orientale* are left out unpaired. This means allosyndetic pairing between 8 chromosomes of *prostratum* with 8 of *orientale*.

If it was allosyndetic, then all the 13 chromosomes of *orientale* must have paired with 13 *prostratum* chromosomes (Drosera scheme). It cannot be that 8 alone of *orientale* chromosomes could be homologous with 8 chromosomes of *prostratum* and the rest did not show any homology. So it is likely that the 8 bivalents frequently met with are the result of autosyndetic pairing among the *prostratum* chromosomes. This means that there is no pairing at all between *prostratum* and *orientale* chromosomes.

From the above two suggestions, two things are evident. (1) That the haploid sets of *orientale* chromosomes are not sufficiently homologous with one another to pair among themselves. So Til might have arisen through allopolyploidy. (2) An absence of pairing between the two parental chromosomes sets indicates the lack of homology between the chromosomes of *prostratum* and *orientale* and hence they could not have had a common origin.

This absence of homology as indicated by absence of autosyndesis amongst the *orientale* chromosomes must be due to either of two causes: (1) That the basic number of Til is 13 and that polyploidy has not played a part in the evolution and that the 13 gametic chromosomes, even though they may not show wide disparity in their morphology, are none the less structurally different from one another which results in their non-pairing. (2) The second alternative is that it should have arisen from a lower chromosome-numbered ancestor through the operation of polyploidy. If so, the absence of autosyndesis indicates that allopolyploidy has been the operating factor. Of these two alternative possibilities the latter seems the more likely.

In the previous investigation as well as in the present, the phenomenon of secondary association has been frequently met with and on the basis of maximum association, it has been suggested that 7 is the basic number of the species—meaning thereby that Til must have arisen from an ancestral form with a set of 7 haploid chromosomes. How this 26 chromosomed Til could have been evolved from such a basic number, through the operation of allopolyploidy, can be explained as follows:—

Supposing there were 2 ancestral species P_1 and P_2 each having 7 haploid chromosomes, one parent from P_1 would have gametic genome A, B, C, D, E, F and G, whereas the other parental form P_2 possibly arisen through gene mutations, not involving numerical change, would have a genome of the same number of chromosomes (7), A_1, B_1, C_1, D_1, E_1, F_1 and G_1. Then the parents are:

P_1	A	P_2	A_1
	B		B_1
	C		C_1
	D		D_1
	E		E_1
	F		F_1
	G		G_1

Gametic genomes. n: 7.

A natural cross between the two forms P_1 and P_2 would result in a hybrid P_3 having:

P_3	A	A_1	
	B	B_1	
	C	C_1	
	D	D_1	$2n$: 14. Sterile hybrid.
	E	E_1	
	F	F_1	
	G	G_1	

The hybrid P_3 is presumably sterile because chromosomes A and A_1 from parents P_1 and P_2 are not homologous and so do not pair.

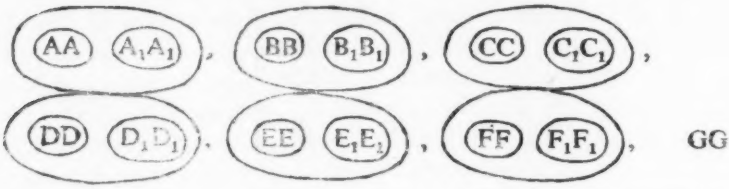
Somatic doubling takes place by some means, say, fusion of unreduced gametes. Then the chromosome sets in P_3 will be doubled resulting in P_4 having $2n$: 28.

P_4	AAA_1A_1
	BBB_1B_1
	CCC_1C_1
	DDD_1D_1
	EEE_1E_1
	FFF_1F_1
	GG (G_1G_1) Deletion.

Deletion of one pair of duplicated chromosomes in P_4 results in the disappearance of one of the chromosome pairs say (G_1G_1). Now P_5 resulting after the deletion of one pair would contain $2n$: 26 only (= *S. orientale*).

P_5	AA	A_1A_1	
	BB	B_1B_1	
	CC	C_1C_1	
	DD	D_1D_1	$2n$: 26
	EE	E_1E_1	
	FF	F_1F_1	
	GG	—	

Meiosis in P_5.—13 pairs are formed in 7 groups. Because of distant homology of A and A_1 chromosomes AA bivalents is secondarily associated with A_1A_1. Thus we get 6 groups of secondarily associated bivalents, *viz.*,



GG remains unassociated, G_1G_1 having been deleted during separation.

During the disjunction in P_5

A	is separated from	A	A_1	is separated from	A_1
B	,,	B	B_1	,,	B_1
C	,,	C	C_1	,,	C_1
D	,,	D	D_1	,,	D_1
E	,,	E	E_1	,,	E_1
F	,,	F	F_1	,,	F_1
G	,,	G			

So the gametic genome consists of:

A and A_1, B and B_1, C and C_1, D and D_1, E and E_1, F and F_1, & G. Since A and A_1, B and B_1, C and C_1, D and D_1, E and E_1, F and F_1 are not homologous enough to pair, as evidenced in the non-pairing in P_3 leading to its sterility, they do not show autosyndesis in the meiosis of the sterile hybrid ($2n$: 29)—*Sesamum orientale* (13) × *Sesamum prostratum* (16).

The fact that these remained unpaired without any autosyndesis therefore implies an allopolyploid origin of Til in the manner suggested above.

So far no species of *Sesamum* having n: 7 has been recorded. It is reasonable to expect such wild forms to be putative ancestors to the cultivated form. Only an extensive search for the wild ancestors of cultivated forms, such as organised by the Soviet, can throw light upon the problem.

(*e*) *The possible origin of Sesamum prostratum* Retz.

It is interesting to note that while one set of parental chromosomes (*Sesamum orientale*) do not pair among themselves, the other set of parental chromosomes (*Sesamum prostratum*) pair autosyndetically. The cause for the non-pairing among the *orientale* chromosomes is likely to be its origin as an allopolyploid in the manner previously described.

On similar lines, it may be supposed that autosyndetic pairing of *Sesamum prostratum* chromosomes in the sterile hybrid might be due to its origin as an autopolyploid from an ancestral form having haploid chromosome number

8. If that be the case then the ancestral form will have the somatic constitution as

AA, BB, CC, DD, EE, FF, GG, HH } $2n$: 16

Supposing doubling of chromosomes takes place then the zygote will contain $2n$: 32

AAAA, BBBB, CCCC, DDDD, EEEE, FFFF, GGGG, HHHH } $2n$: 32

Presumably no quadrivalent formation takes place and 16 bivalents are formed during the meiosis in *Sesamum prostratum.*

Since A and A are homologous, they pair and hence when they are in a new surrounding, namely, with *orientale chromosomes* in the sterile hybrid, they pair among themselves. They do not pair with *orientale* chromosomes because they are structurally different from them. This means that *Sesamum orientale* and *Sesamum prostratum* could not have arisen from a common ancestor. For, if that were so, the *Drosera*-scheme of pairing would have been exhibited in the hybrid meiosis. If the frequent occurrence of 8 bivalents could mean autosyndetic pairing of 16 chromosomes of *prostratum* chromosomes, then it is likely that *Sesamum prostratum* has arisen as an autotetraploid from a parent having 8 haploid chromosomes in the manner described above.

In the meiosis of *Sesamum prostratum* there has been observed a regular absence of multivalents. Normally in autotetraploids, any four chromosomes ordinarily tend to form a quadrivalent group in meiosis. Often the synaptic association is such as to group the four members into two bivalents. Thus tetraploid sporocytes may sometimes exhibit the diploid number of bivalents " the double diploid " (Sharp, 1934).

According to Crane and Lawrence (1934) it seems that competition in pairing at prophase meiosis in an autotetraploid may give rise to univalent chromosomes instead of multivalents.

Autotetraploids may change the pairing habit of their chromosomes and the number of chiasmata may be reduced to one for each chromosome so that no quadrivalent formation can be formed (Darlington, 1939). In *Tulipa* tetraploids this is found to happen to a varying degree (Margaret Upcott, 1939). It is observed that the chiasma frequency of the tetraploids is low, are sexually reproducing, and have been subjected to selection because of their origin from diploid ancestors. They have been selected for fertility and hence the absence of multivalents.

Many species have been found to include a series of polyploid forms. In some cases these are indistinguishable from one another except by distribution. It is plausible to assume that these forms have arisen as autopolyploids with free pairing amongst their homologous chromosomes. This condition is still found in certain forms which have presumably remained unaltered since their origin.

However, in most cases of autotetraploids low fertility or complete sterility has been the rule. This is due to the irregular meiosis. Formation of multivalents is very common and their disjunction is unequal. Hence polymorphic grains are formed which are non-viable. So in speciation, autopolyploidy has not played as important a part as allopolyploidy. There are however cases where autotetraploids have established themselves as stable species. They show regular meiosis and bivalents have been found instead of multivalents. Upcott (1939) has recorded tetraploids showing no multivalent formation in *Tulipa*-species. The autopolyploids of *Tradescantia* (Anderson and Sax, 1936) is another instance in point. The above authors have reported the occurrence of an entire group of vigorous autopolyploids in the genus *Tradescantia*. These unlike the usual autopolyploids were found to reproduce themselves by seeds.

Sesamum prostratum may well be included under such autotetraploids. The perennial habit, the luxuriant growth and the high yield mingled with the non-susceptibility to any disease, either fungal or insect, may be an additional advantage acquired by *Sesamum prostratum* through autotetraploidy. According to Erlanson (1938) polyploid forms are better fitted to withstand Arctic or Alpine conditions while the diploids will simply perish. Navaschin (1929) has pointed out that " through changes in the rate of development, a polyploid individual may acquire the ability of withstanding different climatic conditions, and as a consequence, penetrate into new territory ". Hagerup (1933) also has stated that " polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid forms will not thrive".

If *prostratum* could have arisen through autopolyploidy and established itself as a stable form, then the presence of other wild forms like *Sesamum radiatum* Shum and Thonn (n: 32), *Sesamum laciniatum* Klein (n: 14) may be explained as a series of polyploid forms arising out of the putative ancestor having n: 8. Then *Sesamum radiatum* (n: 32) will be an octoploid whereas *Sesamum laciniatum* (n: 14) will be a tetraploid, having lost a pair of chromosomes in its meiotic complement (from n: 16 to 14). Morphological evidences also may add proof to the inclusion of these two wild forms in the scale of polyploidy. *Sesamum laciniatum* and *Sesamum radiatum* have been found to grow luxuriantly maintaining at the same time the perennial habit. So, it might be that these forms, along with *Sesamum prostratum*, have arisen from an ancestor having a basic number of chromosomes n: 8, as autopolyploids.

V. Summary

Interspecific crosses between *Sesamum orientale* Linn. and *Sesamum prostratum* Retz. were effected reciprocally and the sterile hybrid was made fertile by artificial induction of amphidiploidy through colchicine. The cytology of the parents and the hybrids was studied in detail.

Details of meiosis of *Sesamum orientale*, one of the parents employed have been worked out. The peculiar persistence of the nucleus and its movements during the meiotic cycle are recorded. The other parent *Sesamum prostratum* has also been cytologically studied.

The irregular meiosis of the sterile hybrid and the occurrence of scattered bivalents and univalents in the metaphase plate, leading to the ultimate formation of abnormal sporads have been described fully.

The regular meiosis of the fertile amphidiploid is compared with the irregular meiosis of the sterile hybrid and the cause of this regularity is explained.

The nucleolus with behaviour of the special regard to its persistence and movements is discussed.

Interspecific hybridisation as a guide to ancestral homology and the artificial synthesis of a new species are discussed in the light of cytological data gathered in the present investigation.

The origin of the cultivated Til *Sesamum orientale* Linn. from a putative ancestor having haploid number 7 through allopolyploidy is traced with the help of cytological details obtained in the hybrid meiosis.

The origin of the wild *Sesamum prostratum* Retz. is also traced to an ancestral form possessing haploid number of 8 chromosomes through autopolyploidy.

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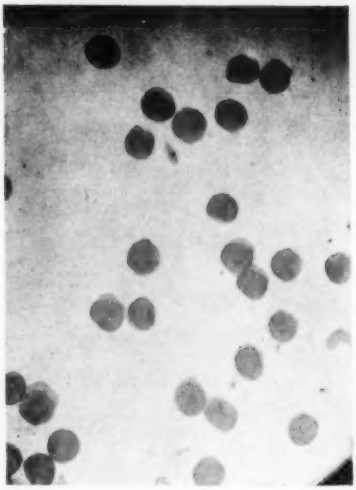

FIG. 1

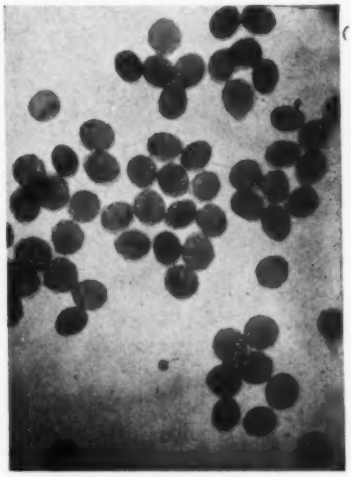

FIG. 2

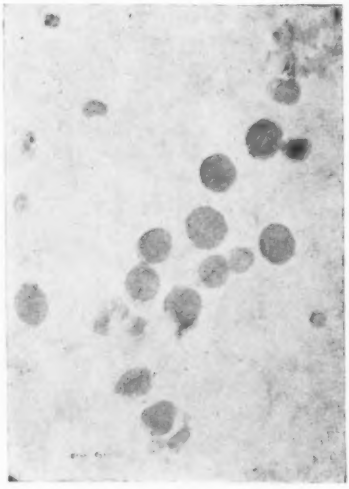

FIG. 3

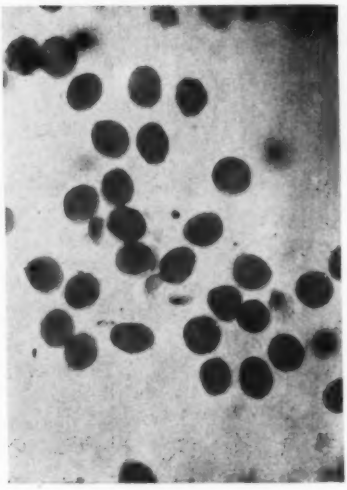

FIG. 4

Microphotographs of pollen grains of the parents and hybrids

FIG. 1. *Sesamum orientale* Linn. Uniform
FIG. 2. *Sesamum prostratum* Retz. Uniform
FIG. 3. Grains of the sterile hybrid. Polymorphic
FIG. 4. Grains of the Fertile amphidiploid. Big and uniform

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